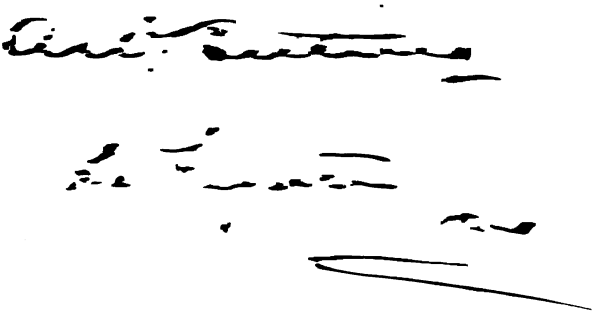
The Bacterial Poisons

By Dr. N. Gamaleïa.



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THE

Bacterial Poisons.

BY

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GEORGE S. DAVIS,
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PREFACE.

The Bacterial Poisons are here for the first time made the subject of a connected treatise. The general plan of this work is as follows:

When bacteriology, extending its domain beyond certain etiological data concerning infection, sought to penetrate the pathogeny thereof, there arose a necessity of undertaking the study of chemical poisons produced by the microbes. This study first imposed itself in diseases such as cholera, diphtheria, and tetanus, where the pathogenic agent is found lodged in a limited part of the organism and yet causes a general affection. In these cases, the pathogenic action of the bacteria can be explained only by the systemic poisoning produced by the specific products of these bacteria. But the same explanation has been found to hold good in diseases of another type, where, as in tuberculosis, the lesion produced by the pathogenic agent is strictly circumscribed to the spot where this agent vegetates; here also we have proved that the lesion does not come from the microbe as a living organism, but from its chemical poisons—the lesion and the disease may be reproduced by the dead microbe, which can, of course, act only by the toxic substances which it contains.

The study of bacterial poisons concerns not only the pathogeny of infectious diseases; it finds also practical applications for their prophylaxis and treatment. It has been demonstrated that a sure and inoffensive vaccination may be obtained without the help of living microbes, and that the refractory state may be conferred simply and solely by the soluble products of micro-organisms. Lastly, a new method has been found—immunization—which has direct applica-

tions in therapeutics, being capable not only of preventing disease, but of curing it when once declared. Here the rôle of living bacteria is even less than in chemical vaccination. The cure of infectious diseases is obtained with the serum of vaccinated animals.

The theory of immunity has also profited by the knowledge of the poisons of bacteria; it has been shown that the immunity of animals against infectious diseases is in close relation with their resistance to microbial poisons; the latter is subordinated in its turn to the elaboration by these animals of special substances—antitoxines—which have the property of neutralizing the action of microbial toxines.

From this aggregate of facts, a new science has sprung up: the science of Microbial Poisons, which is based at once on bacteriology, on biological chemistry, and on general physiology.

From bacteriology it borrows its data about microbes as producers of poisons; but the important results obtained with the analogous poisons of different origin, such as abrine, ricine, robine, necessitate the extension of the domain of microbial toxicology beyond microbism.

To biological chemistry belong the methods of preparation and of isolation of bacterial poisons; from it we must demand information as to their nature and their production.

Lastly, the study of their mode of action on the animal organism, of their diffusion, and of their destruction in the organism, belongs to the domain of general physiology.

Thus, microbial toxicology touches at once on bacteriology, on biological chemistry, and on general physiology; it is the science of bacterial poisons, and has for its object the study of their chemical nature, of their mode of production, and of their action on animals.

This work is divided into three parts:

The first part — History of Microbial Toxicology — de-

scribes the evolution which the study of systemic poisoning in the infections has undergone.

The second part—General Toxicology—treats of our actual information respecting the chemical nature of the microbial poisons, their production, and their destiny in the animal body.

The third part—Special Toxicology—will be the exposition of the data acquired concerning the toxines of different diseases.

N. G.

Paris, May, 1892.

THE BACTERIAL POISONS.

First Part: History of the Development of Our Knowledge Respecting the Bacterial Poisons.

CHAPTER I.

EXPERIMENTAL STUDY ON THE PUTRID POISON.

SUMMARY.—*Experiments with the Toxic Effects Produced by Substances Undergoing Decomposition—Seybert, Gaspard, Stich Establish the Symptoms and the Lesions of Experimental Septicæmia—Panum Gives the Proof that it is Produced by a Chemical Poison—Bergmann and Schmiedeberg Determine This Poison to be an Alkaloid: Sepsine.*

The first microbial poisons studied experimentally were products of putrefaction. Chemists were led to this study by the important etiological rôle which putrefaction plays in ancient medicine. It was believed that the emanations from decomposing matters might produce typhoid and malarial fevers. The

putrefaction of wounds gave rise to the complications so formidable and so frequent, of septicæmia and pyæmia. A "spontaneous" tendency of the living organism to putridity characterized certain widespread diseases—such, for instance, as scorbutus. The diseases of putrid origin thus constituted an important group of affections which corresponded nearly to our infectious diseases of to-day.

Despite this importance which, from an early antiquity, medicine accorded to putrefaction, the experimental study of the latter is relatively recent.

Towards the end of the last century, Seybert experimented with blood serum, pus, and putrid meat. He introduced these matters into the general circulation of the dog. He noticed that the toxic effects varied proportionately to the quantities of liquid injected. Thus, for instance, 20 cubic centimeters of putrid serum killed a dog in the space of several hours, with vomitings, convulsions, and progressive enfeeblement; 3 grammes caused death in two days, with dysenteric phenomena; doses below two grammes produced only a temporary diarrhœa, followed by a complete restoration of the animal. Seybert also studied the effects of the same putrescent substances when they were introduced by mouth into the stomach. In these cases the toxic effects were *nil*. Moreover, in opening the stomach of the dog three hours after a meal of putrid meat, Seybert no longer found in the mass undergoing digestion the

putrid aspect and odor. Seybert cites in this connection the researches of Spalanzani, who was absolutely convinced of the innocuousness of putrid foods after having fed dogs, cats, and birds with them.

Gaspard, physician of Saint Etienne, pursued some experimental researches on the putrid poison. His experiments established several important points respecting the effects of putrid matters.

Gaspard showed that decomposed blood, pus, and meat produce in animals a rapid and mortal intoxication. On the contrary, fresh organic liquids such as saliva, urine, and sperm, when injected for control, are found to be non-toxic. Gaspard showed, moreover, that the putrid infusions of plants also poison animals.

The symptoms of this poisoning were very constant and typical, being independent of the source of the putrid substances, whether vegetable or animal, and of the place of the injection. This intoxication (septic or putrid infection) was characterized by tremblings and convulsions, vomitings, and diarrhœa, the latter often sanguinolent. The animals died in dyspnœa, cyanosís, and complete prostration.

At the autopsy, the pathognomonic lesion of the putrid infection consisted in a hæmorrhagic enteritis.

Gaspard concluded from his experiments that a special poison, the *putrid poison*, is formed by putrefaction. This poison produces the same disease and the same lesions in all the animal species.

In order to determine the nature of the putrid poison, Gaspard experimented with the different gases formed by putrefaction. He found that carbonic acid and sulphuretted hydrogen are not toxic; that ammonia, on the contrary, is toxic, but that it cannot produce the symptoms of putrid infection. Hence was overthrown the preconceived idea of the ancients, who believed the principal danger to be in the putrid gases of decomposition. The researches of Gaspard established subsequently the formation, in the course of putrefaction, of a special and very active poison.*

Other experimenters followed in the way marked out by Gaspard. Magendie, Leuret, Dupuis, Darcet, and Sedillot confirmed on all the principal points the fundamental reseaches of Gaspard. They discovered his putrid poison, without, however, determining its chemical nature.

Virchow found that the putrid poison does not act, like the other chemical poisons, in proportion to its quantity. Its activity depends rather on the degree of decomposition of the substances whence it is derived. From this point of view, Virchow ranks it among the ferments.

Stich has made very careful and interesting researches on putrid matters. He injected putrefied albuminoid products, after repeated filtration through

*Gaspard: Physiological Memoir on the Purulent and Putrid Maladies (Journal de Physiologie, 1822 and 1824).

paper, into the veins of dogs, of hares, and of birds. The latter showed themselves the most susceptible to poisoning. The typical lesion consisted in a hyperæmia of the intestines.

But especially interesting are the researches of Stich on the toxicity of fæcal matters. The aqueous extract of the excrement of a dog, injected into the veins of this animal, was found to be very toxic; but the same extract introduced into the stomach or rectum of dogs was completely inoffensive.

Stich carried this study farther. Introducing the excrement of one animal into the stomach or rectum of an animal of another species, he found that poisoning was the result. It follows that immunity exists for animals only in relation to their own intestinal contents. Stich asks: What are the causes of this absence of auto-intoxication?* He leaves the question unsolved.

The most important researches on the putrid poison belong indisputably to Panum.

Panum asks if the accidents of putrid infection are really due to a chemical substance or to the action of bacteria multiplying in matters undergoing putrefaction? To solve this question, he instituted numerous important researches on the putrid poison, and confirmed the results obtained by Gaspard. He produced a typical intoxication by the injection of putrefied meat. This intoxication was characterized by

*Stich, Charité Annalen, 1853.

prostration, vomiting and diarrhœa, and rapid death. The autopsy showed a more or less pronounced gastro-intestinal catarrh.

Having reproduced this putrid infection of Gaspard, Panum then demonstrated that it was due to the action of a chemical poison independent of the agency of bacteria. The putrid liquids, though perfectly clear, and freed from every foreign germ by repeated filtration through paper, none the less retained their toxic properties. Nor was the putrid poison destroyed by ebullition for eleven hours, which ought surely to kill all living germs. Having thus settled the fundamental question as to the inorganic character of the putrid poison, Panum studied its chemical properties. He found that this poison was not volatile; for the distilled products of the putrefied matters, while having the fetid odor, are not at all toxic. The putrid poison was fixed, and the dry residue of the evaporation of the putrefied matters retained all their toxic properties. This dried residue did not give up to alcohol its chief toxic principle. The alcoholic extract had a different physiological action from the putrid matters; it produced a manifest narcosis, which Panum compared to that produced by the alkaloids of opium. The veritable putrid poison capable of causing the typical poisoning was, on the contrary, soluble in water, although it adhered to the precipitates of the albuminoid substances coagulable by heat; it was also partially retained by the filters.

Panum showed also that his poison had nothing in common with the well known substances produced by putrefaction, such as leucin and tyrosin. He stopped here in his chemical investigation, and did not further indicate the nature of his putrid poison. It was sufficient for him to have given the irrefutable proof that it was not organized.

The work of Panum produced an enormous impression upon the scientific world. Hidden by its author in an unknown Danish publication, the memoir of Panum was found there by a German writer, who published an analysis of it in Schmitt's Jahrbücher for 1859. In this extensively circulated publication the results of Panum's researches became known to the whole scientific world. A great number of experimenters in Germany and, especially, in Russia, occupied themselves with the verification of these results and the chemical study of the putrid poison.

The University of Munich then offered a prize for the best essay on "Putrid Infection and Its Causes." Two memoirs were deemed worthy of a prize: those of Henner and Schweninger. These writers confirmed the fundamental result of Panum, that the putrid poison is of chemical nature, and that the action of microbes is *nil* in putrid infection. As to the nature of the poison, they favor Virchow's view that it should be ranked among the ferments, for it acts in infinitesimal quantity, has a period of incu-

bation, and always produces the same typical affection.

Since the same year, 1866, there have appeared at Dorpat numerous theses devoted to the chemical study of the putrid poison. We may cite the memoirs of Raison, Frèse, Weidenbaum, Schmitz, Petersen, A. Schmidt, and Brehm. These theses also confirm Panum's position. Raison shows that the putrid poison is not retained by filtration through charcoal. The minimum dose of 0.0036 gramme of the filtered liquid killed a horse by intravenous injection. He found also that the putrid poison, evaporated to dryness, could be subjected for several hours to a heat of 130° C. Weidenbaum has seen that the putrid poison supports discontinuous ebullition, repeated several times. These two writers, and the other pupils of the school of Dorpat whom we have mentioned, have also seen the putrid poison support without alteration the different chemical manipulations, such as the action of sulphuric and hydrochloric acids, precipitation by acetate of lead and nitrate of mercury, etc. Lastly, they even succeeded in obtaining the putrid poison in a state of purity, and of determining its chemical nature. It is the celebrated sepsine found in 1868 by Bergmann and Schmeideberg. This important result was accomplished by a complicated method based on the employment of corrosive sublimate.

In 1866, Bergmann published the results of his

researches made with Schmiedeberg on the chemical properties of the poison contained in putrefied yeast. They found that this poison supports for eight hours digestion in boiling alcohol. The alcoholic extract of the putrefied yeast is found, after evaporation, to be powerfully toxic. To separate the active principle, Bergmann and Schmiedeberg precipitated it in alcoholic solution by corrosive sublimate. The mercurial precipitate was washed in alcohol, dissolved in water, and decomposed by hydrogen sulphide. The liquid, freed from sulphide of mercury by filtration, was heated to get rid of the excess of hydrogen sulphide, and treated with carbonate of silver, which, by forming an insoluble chloride of silver, removed free hydrochloric acid from the solution. The filtered liquid obtained after this last operation was almost colorless and perfectiy clear. It had the specific toxicity of the putrid poison.

In 1868 these experimenters (Bergmann and Schmiedeberg) obtained, in crystalline form, the salt of this poison with sulphuric acid, and called it the sulphate of sepsine. This salt was proved to be eminently toxic. Injected in the dose of one centigramme in the vein of the dog, it provoked immediately vomiting and sanguinolent diarrhœa, and at the autopsy hæmorrhagic ecchymoses were found in the stomach and intestines.

The publication of Bergmann and Schmiedeberg's discoveries naturally produced a profound impres-

sion. For a time, chemists believed they had at last got hold of the famous putrid poison whose existence had been demonstrated by Gaspard, whose purely chemical nature had been proved by Panum, and which had finally been obtained in a crystalline form by Bergmann and Schmiedeberg. This sepsine would alone henceforth explain all the various accidents of medical and surgical septicæmia. It was believed that the way was finally opened for the easy interpretation of all the infections. Chemists went to work to seek out and to study this sepsine. Petersen and A. Schmidt found it in putrid blood, and thus confirmed the data of Bergmann and Schmiedeberg.

Nevertheless, the most competent authorities soon admitted the impossibility of ascribing all the effects of the putrid poison to sepsine alone. First, it is not found in all putrefied substances; thus, for instance, Fischer could not find it in putrid pus. Bergmann himself, in applying his method of extraction to different putrid matters, has isolated substances which had nothing in common with sepsine. Other toxic substances were extracted from the putrid matters.

Zuelzer and Sonnenschein have found another septic alkaloid in the infusions of putrid meat. This body has all the reactions of the vegetal alkaloids, such as atropine and hyoscyamine. It possesses also the physiological action of the latter. Injected under the skin of animals, it provokes dilatation of the

pupils, relaxation of the intestines, and exaltation of the heart's action.

The conclusion was inevitable that the poisons produced by putrefaction were numerous and different, according to conditions necessary to determine. In the following chapter we shall study the researches which have been undertaken to elucidate the rationale of putrefaction and of septicæmia.

Only, we shall see in studying the different possible causes which influence the production of the putrid poison, that experimenters had overlooked the principal one, viz., the intervention of microbes. Between the Chemical School and the Vitalists, there was destined to be a memorable struggle, which ended in the victory of the latter—a victory which completely cast into the shade the results acquired by the chemical study of the putrid poison.

CHAPTER II.

THE MICROBIAL ETIOLOGY OF PUTREFACTION AND OF THE INFECTIONS.

SUMMARY. — *Contest between the Chemical and the Vitalist Doctrines—The Contradictions and the Results Obtained by the Chemists—Their Explanation Insufficient by Reason of the Absence of the Notion of Specificity—Definitive Victory of the Vitalist Doctrine—The Infectious Diseases are Admitted to be Caused by Specific Fermentations.*

From antiquity, two contrary interpretations of the etiology of putrid and infectious diseases have prevailed.

The Chemical Doctrine set forth in the previous chapter is, that matters in the process of decomposition constitute poisons sufficient to account for all the symptoms of the infections, and that the microbes found in these putrid matters are only inconstant and inoffensive accompaniments. The other, or Germ Theory, affirms that the microbes are really the sole agents of putrefaction and of infectious diseases, and that just as the inanimate matters do not alter and ferment without the intervention of microbes, so the presence of the latter is indispensable for the produc-

tion of the infectious disease. We shall now see how this Vitalist doctrine has definitively supplanted the Chemical.

The chemical and toxicological researches on the putrid poisons did not realize the fundamental condition of all scientific work; they did not give results that were always constant and uniform. Chemical analysis revealed different properties in the active principles isolated. Experimentation in the hands of different chemists did not produce the same symptoms and the same lesions with the poisons obtained from putrid substances. This inconstancy of results was soon noticed by investigators. Thus, for instance, one of them * expresses himself in this way:

" There is not agreement as to the pathology of the putrid infection or as to its nature. Thus, certain chemists (*Henner*, *Schmidt*, *Petersen*) affirm that the convulsions and tetanic attacks are important characters of putrid infection; others, on the contrary (*Dupuis*, *Sedillot*, *Stich*, *Raison*, and *Schmitz*), have never or but rarely seen them. Even the constant existence of vomiting and diarrhœa, supposed to be pathognomonic of putrid infection, is denied by certain experimenters. The same may be said of the anatomical lesions. Thus *Gaspard*, *Leuret*, *Virchow*, *Panum*, *Stich*, *Henner*, *Bergmann*, and others affirm

* Rawitsch: Zur Lehre von der putriden Infection und deren Beziehungen zum sogenannten Milzbrand. Berlin, 1872.

that hæmorrhagic inflammation of the intestinal mucosa is a characteristic lesion; while others (*Dubuis, Sedillot, Billroth, Raison,* and *Schmidt*) have found in mortal cases the intestinal mucous membrane intact. Several writers think that sanguineous ecchymoses in the different organs are characteristic of putrid infection, while others have not seen them in their autopsies. Bergmann believes that the modifications of the spleen are constant and pathognomonic, while Davaine affirms that it is just the absence of hypertrophy of the spleen in putrid intoxication that enables us to differentiate it from charbon.

To explain the causes of these variations in the results of experimentation, writers have referred us to the different sources of the putrid substances, alleging that putrefied meat acts differently from putrefied blood, etc.—Stress has also been laid on the differences of the action of the putrid poison, according to the different places of its introduction into the animal economy. Thus, the effects of subcutaneous injections were distinguished from those of intravenous injection; in the first case, a more or less intense local inflammation was aroused, complicated with suppuration and gangrene; in the second case, there appeared the septic infection, as Gaspard described it.—We have, moreover, been referred to the different receptivity of the animals under experimentation, varying according to species, race, and age.—But it has been especially remarked that the toxicity of

putrid products undergoes very considerable variations in the course of putrefaction. The putrefied matters, instead of becoming more and more toxic as decomposition went on, were found to be even less toxic, the first stages only giving rise to the most violent poisons. Billroth shows that relatively fresh pus, even when it is not putrid but "good and laudable," may produce by intravenous injection very violent and febrile reactions, even ending in death. H. Fischer demonstrated that pus which was very toxic at the onset, loses, in putrefying, its specific and septic toxicity. Bergmann has found that putrefied blood is more toxic in the first five to six days. Heller has remarked the same fact.

Samuel has studied this question in detail. He finds that there are three periods in the toxicity of putrid substances: phlogogenous, septogenous, and pyogenous. In the first, the inoculation of putrid substances is followed by transient inflammatory symptoms which disappear without leaving any local or general troubles. In the septogenous stage, specific toxic products appear which provoke the true "septic infection." It is here that we note the most terrible local and general phenomena of septicæmia: the former going on to septic gangrene, the latter to fulminant septicæmia which kills with the rapidity of hydrocyanic acid. Later on, these septic properties of the progressively decomposing putrid substances become attenuated little by little, to leave remaining

in the pyogenic stage only the power of prodncing localized and benign suppurations.

But all these distinctions, despite their considerable interest, did not quite suffice to explain the inconstant and conflicting results of experimenters.

There remained always an irreconcilable difference between the chemical poison of Panum, the activity of which depended on the dose injected, and the virus of Davaine, which acted in infinitesimal doses and which was found in increased quantity in the cadavers. It was evident that the septicæmias of which the different writers spoke were not at all identical, and that their differences depended on a factor having quite other powers than those alleged by the chemists. But above all, the chemical doctrine was unable to explain the remarkable fact discovered by Coze and Feltz, and confirmed by Davaine, Hiller, and others: the septic poison, instead of being enfeebled by dilution, like all the chemical poisons, in the cadavers of the animals it had killed, was found to be exalted. It was evidently a special poison fitted to reproduce itself just like living beings. From this fact there was only one step to the conclusion that this poison was constituted by bacteria. One might, it is true, still find refuge in the hypothesis of Liebig and Robin, that the soluble ferments are albuminoid matters in process of decomposition, which may transmit their alterability to other bodies and reproduce themselves in this way indefinitely. But this hypothesis

of Liebig was completely ruined in its chemical applications by Pasteur, who demonstrated peremptorily that fermentation (or decomposition) does not exist apart from the life of organized ferments. Moreover, the contagionists were soon able to go farther in showing the fallacy of the chemical doctrine, and to prove that certain microbes separated from their morbid products were able to produce determinate diseases. Thus, it was proved that charbon, heretofore ranked among the putrid fevers, was produced by the bacterium of Davaine; that septicæmia was caused in man by the septic vibrio of Pasteur; that the different septicæmias and pyæmias in animals had each its special pathogenic agent, living staphylococci or streptococci. At the same time was established the fundamental notion, proclaimed by Ferdinand Cohn, of the specificity of the bacteria, which constitute each a being apart, characterized by its morphology and by its functions, chromogenic, zymotic, or pathogenic. Weigert and Ehrlich brought to the aid of this study the processes of staining by anilin dyes, which enable microscopists to recognize the bacteria. Koch introduced the Abbé method of illumination and the method of culture on solid media, which have helped bacteriologists to make immense strides in their knowledge of the microbes. Thus was constituted Bacteriology, which has been making such rapid advances. It is now known with certainty that the infectious diseases are due to the action of microbes.

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The vitalist doctrine was not in absolute contradiction with the results obtained by chemists, and Panum has the merit of having understood, just as Selmi and Brieger did later, the possibility of reconciling the two theories.

Panum said "that it is incontestible that my putrid poison is purely chemical, but it might be produced by a microbe, even a determined microbe. In diseases, both the pathogenic microbe and the production of the poison by the microbe may play their respective parts."

But the conciliatory voice of Panum was not heeded, and the chemists made vigorous opposition to the victorious march of bacteriology. They continued to bring forward proofs in favor of this view—that the microbes were but simple accompaniments of the poisons of Panum, Bergmann, and Schmiedeberg. They filtered the toxic products through filtering paper or through clay, and studied the effects of these filtered liquids. They employed heat, dialysis, the different chemical reagents, to eliminate the living microbes of their experiments. They cultivated the microbes in media deprived of albuminoid substances, to show that in these cases they were inoffensive (Hiller). But to bacteriologists all these experiments of the chemists were null and void, for they could not have been well conducted without previous knowledge of the properties of the bacteria. Nay, more; all the experiments on systemic intoxication where the in-

fluence of a chemical poison made itself felt, could but embarrass the bacteriologists, adding a supplementary factor to their study of the functions of bacteria.

Under these conditions the bacteriologists, quite naturally, made a profound distinction between intoxication and infection. Intoxication was characterized by the sudden appearance of the accidents, by the proportionality of the effects to the doses (doses always considerable), by the absence of specificity—for it could be produced by the most common bacteria if injected in sufficient quantity. It had to be carefully avoided in bacteriological studies. These concerned themselves only with *infection*, which was produced by life and the multiplication of specific microbes in the animal body. It was independent of the dose of microbes introduced into the animal body, and it appeared after an incubation necessary for their multiplication. The infectious disease was a specific fermentation, linked to the life and multiplication of the pathogenic microbes, just as alcoholic fermentation depends on the life of the yeast.

In ignoring intoxication the bacteriologists had to ignore all the experimental researches on the chemical poisons; and the dualism of intoxication and infection long bore as a heavy weight on the development of microbiology. But during this time the microbian poisons reappeared in a quite different science, and their study was being pursued outside of bacteriology.

CHAPTER III.

THE DISCOVERY OF PTOMAINES.

SUMMARY.—*Selmi Draws Attention to the Ptomaïnes Obtained from Cadavers—Leucomaïnes and Ptomaïnes of Gautier—The Researches of Brieger on Ptomaïnes.*

In a celebrated trial, where the domestic of Gen. Gibbone was accused of the death of the latter, the chemists found in the cadaver the alkaloid *delphinine*. But Francois Selmi, professor of chemistry at Bologne, who was called as an expert witness for the defense, demonstrated by a detailed chemical analysis that the base isolated by the government experts was quite distinct from the delphinine and other alkaloids of vegetal origin. According to Selmi, the base in question was of animal origin, and came from the putrefaction of the cadaver. This dictum of Selmi was completely at variance with the general conviction, according to which the alkaloids could only be produced by plants. Shortly after, similar facts were adduced, and drew attention more and more to the researches of Selmi. In the trial of Sonzagno, at Cremona, the first experts thought they had found morphine in the cadaver. Selmi peremptorily demon-

strated that what they had found was a cadaveric alkaloid.

There was still later a celebrated trial in Italy where the prosecution endeavored to make out a case of poisoning by strychnine; here Selmi affirmed the cadaveric source of the base found, and the prisoner was acquitted on this testimony.

Since 1870, Selmi has been making experimental and chemical investigations on the organic bases found in cadavers. To this order of researches he was led by noting the presence in cadavers of substances which possessed the general and even the specific reactions of alkaloids, but which could be distinguished by the absence of all toxic action. Selmi had the notion that alkaloids similar to those of animal origin could be produced by the very fact of putrefaction. To verify this idea he instituted a long series of researches upon exhumed cadavers, of which the disease, the death, and the duration of burial were known to him. He there discovered, in fact, a great number of both inoffensive and toxic alkaloids, with different chemical properties, and more or less like the alkaloids of plants.

Pursuing further his researches on these products, Selmi demonstrated their origin in the putrefaction of albuminoid matters, for he found that ptomaïnes also take rise in the albumin of putrefied egg.

These discoveries of Selmi were too revolutionary of former notions to be accepted without opposition.

We have already mentioned how, in certain criminal prosecutions, they forced themselves upon the attention of the whole scientific world.

These expert findings of Selmi in Italy had also their counterpart in Germany, in the celebrated trial of *Brandes–Klebs* in 1874. Chemists had extracted from the cadaver of Klebs a very toxic alkaloid which they had identified with coniine. Otto showed that it was neither coniine nor nicotine, though it resembled these alkaloids. He believed it to be a ptomaïne, and this was the opinion of the experts.

It is doubtless true that Selmi was not the first to find organic bases of animal origin; that Marquardt, of Stettin, in 1865 extracted from a human cadaver a toxic alkaloid resembling coniine, which he called septicine; that Dupré and Bence Jones, in 1868, found in animal bodies a substance bearing a marked resemblance to an alkaloid; that Sonnenschein, in 1869, discovered in a cadaver an alkaloid which he thought was coniine, but which proved not to be toxic; the merit none the less belongs to Selmi of having been the first to subject these animal bases to a profound series of researches, and of thereby explaining their true meaning and importance. It is noteworthy that in Selmi's estimation the interest in the study of ptomaïnes was by no means limited to legal medicine by reason of their simulation of vegetal alkaloids. He had well comprehended the signification which they might have for biological chemistry and physio-

logical pathology. In biological chemistry the ptomaïnes were to Selmi a new demonstration of the absence of any clear-cut limits between the vegetable and animal kingdoms. At the expense of animal matters might be produced alkaloids quite like those which down to Selmi's time were believed to be exclusively of vegetal origin. To general pathology the importance of the discoveries of Selmi was still greater. The same substances which the microbes may produce at the expense of the dead animal body, they may elaborate in the living body; in other words, the infectious disease produced by the microbes may give rise to the formation of ptomaïnes in the diseased organism. In fact, Selmi found ptomaïnes in the urine in cases of typhoid fever, pneumonia, and tetanus. The premature death of Selmi arrested these important researches.

The movement imparted to science by Selmi was of very great importance. A large number of memoirs have been recently published on the ptomaïnes. To mention only a few, Nencki in 1876 gave the first analysis of a ptomaïne, obtained by the putrefaction of gelatin with the pancreas. This was collidine, a base already extracted from the products of distillation of animal oil.

Morrigia and Battistini in 1875 showed that very toxic ptomaïnes can be got from cadavers, whose action is quite similar to that of curare. Rorsch and Fassbender found in the cadaver a ptomaïne resembling

digitalin in its action. Brouardel and Bontmy discovered a base very similar to veratrine. Gautier and Etard, in 1881, defined chemically two other bases, parvoline and hydrocollidine, found in the products of decomposition of fish, and of the meat of the horse and the ox. The fourth ptomaïne defined was *corindine*, found in 1883 by Quareschi and Mosso.

We must devote more time to the extremely important researches of Armand Gautier.

Gautier had announced in 1871 that during the putrefaction of fibrin there is formed a certain quantity of alkaloids, both fixed and volatile. In 1881–1883 he made, with Etard, a series of researches which disclosed the chemical nature of certain ptomaïnes of putrefaction belonging to the pyridic and hydropyridic series.

According to Gautier, all the decompositions of albuminoid bodies taking place away from the air give rise to alkaloids. Putrefaction is always accompanied by the formation of ptomaïnes; but the normal life of animals, which in great part goes on in the absence of free oxygen, leads also to the formation of organic bases, which Gautier has grouped under the name of *leucomaïnes*. Such are xanthine, creatine, etc. Whenever perturbations of the normal conditions of life augment the want of oxygen in the body, as in anæmia and the infectious diseases, then the basic products or ptomaïnes arise in increased quantity. The non-elimination of the normal leuco-

maïnes, and the production of ptomaïnes, are so many causes of intoxication (or poisoning) of the organism. Certain leucomaïnes are extremely toxic; for instance, the secretions of the different venomous animals. (Already, in 1866, Zalewsky had isolated from the salamander the alkaloid *samandrine*.)

We have seen with what amplitude of view Gautier established his conception as to the origin, virtually identical, of the alkaloids in vegetables, animals, and microbes. These bases of the same nature are derived everywhere from the same source, the albumins; everywhere in the same fundamental conditions—decomposition in the absence of sufficient quantity of oxygen. Guided by these general ideas, Gautier and his pupils found a great number of new leucomaïnes. But the exposition of their history does not enter into the plan of this book.

The study of the ptomaïnes properly so called, which interest us particularly here, owes its great progress during the past few years to the researches of Brieger. Brieger has made a systematic study of a great number of questions called up by the history of the ptomaïnes. Thanks to the perfected chemical methods, he has succeeded in isolating numerous ptomaïnes perfectly defined as chemical units. We shall indicate the principal results which he obtained. Seeing the facility with which the ptomaïnes form in the midst of albuminoid substances in putrefaction, Brieger asked if the alkaloids might not result from

the simple solution of the albumins by the soluble ferments. In fact, in digesting meat by pepsin, he noted the appearance among the products of this digestion of a convulsivant organic base, which he called peptotoxine. We shall return later to this remarkable result, which has an enormous importance in connection with this question of ptomaïnes.

In putrid cadavers, and in the flesh of mammals and of putrid fish, Brieger found a certain number of bases regarded by him as constant representatives of the ptomaïnes of putrefaction. They all belong to the category of ammoniacal compounds, amines. Besides the methyl and ethyl amines, Brieger has isolated in a state of perfect purity cadaverine, putrescine, neurine, muscarine, ethylendiamine, saprine, mydaleine, and a great number of others.

They are chiefly diamines. Lastly, Brieger took up again the idea of Panum and of Selmi, and made a study of the ptomaïnes produced by the microbes of infectious diseases. He has studied also the bases formed in the cultures by the typhic and tetanic bacilli, by the vibrio of cholera. But by these latter studies Brieger enters into the new period of the evolution of ideas concerning the rôle of bacterial poisons, a period which shall be set forth in the following chapter.

A. *On the Constitution of the Artificial and Natural Alkaloids.*

The first vegetable alkaloid, morphine, was dis-

covered by Sertürner in 1806. Since then the number of organic bases extracted from plants or prepared artificially has been considerable, especially during the past few years, and chemists have at last succeeded in determining their constitution.

The principal alkaloids are derivatives of pyridine, which much resembles benzol.

Pyridine is a benzol where an atom of nitrogen (N, which is trivalent) replaces the group CH (which has also three free atomicities).

In the formula of pyridine, the atoms of hydrogen may be replaced by the monovalent groups—

$$H-\overset{\overset{C}{|}}{\underset{H}{|}}-H, \text{ or } (CH_3)$$

Thus we have picoline. If this substitution be made twice, we shall have putidine; with three groups of CH_3 instead of 3 atoms of H, we obtain collidine, and after a new substitution, corindine.

All these products are energetic bases obtained artificially by the dry distillation of bones (oil of Dippel). Collidine and corindine are already known to us, for they have also been found in the products of putrefaction.

The natural alkaloids of plants are a little more complex, but they are also the substitution products of pyridine or of piperidine (which is hydrogenated pyrodine).

Thus, coniine is a propylpiperidine, or a piperidine

where an atom of H is replaced by the monoatomic group C_3H_7 which is called propyl. Other alkaloids of plants are derivatives of quinoline, which corresponds to naphthaline where an atom of N replaces the group CH.*

B.—*The Amines.*

Ammonia has the formula:

$$NH_3, \text{ or } \begin{matrix} -H \\ N-H. \\ -H \end{matrix}$$

The atoms of H in ammonia may be replaced by the alcohol radicals. This substitution gives rise to the ammoniacal compounds, or the amines.

Thus, methyl-alcohol has the formula CH_3HO. In this formula CH_3 is the alcohol radical called methyl, combined with the group HO, which is called hydroxyle. The methyl may replace one, two, or three atoms of hydrogen in the ammonia. We shall have monomethylamine, dimethylamine, trimethylamine:

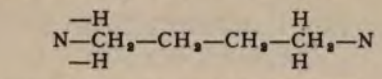
$$\begin{matrix} -H \\ N-H \\ -CH_3 \end{matrix} \qquad \begin{matrix} -H \\ N-CH_3 \\ -CH_3 \end{matrix} \qquad \begin{matrix} -CH_3 \\ N-CH_3 \\ -CH_3 \end{matrix}$$

The bivalent alcohol-radicals may unite with two molecules of ammonia. The resulting bodies are called diamines. Thus, ethylen—C_2H_4—which has the two atomicities free, forms ethylendiamine:

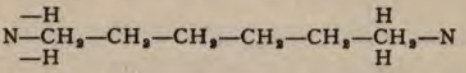
$$\begin{matrix} -C_2H_4 \\ N-HH-N \\ -HH- \end{matrix}$$

* Von Pictet: The Chemical Constitution of the Vegetal Alkaloids. Paris, 1888.

Most of Brieger's ptomaïnes are diamines. Thus, putrescine is a tetramethylendiamine; the bivalent alcohol-radical CH_2 is called methylen:



Cadaverine is a pentamethylendiamine:



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CHAPTER IV.

INFECTION IS AN INTOXICATION BY THE MICROBIAN POISON.

SUMMARY.—*Bacteriology Aims to Explain Infection by the Invasion of the Animal Economy by Microbes—Three Diseases, Diphtheria, Cholera, Tetanus, where this Explanation is Not Possible—Discovery of Chemical Vaccination—Discovery of the Different Chemical Poisons of Bacteria which May Produce All the Symptoms of the Disease without the Intervention of Microbes—Actual Point of View: The Infectious Disease is an Intoxication by the Poison of the Pathogenic Microbe.*

We quit bacteriology at the moment when, obtaining a decisive victory over the chemical etiology of the infectious diseases, it denied the importance of the poisons produced by the microbes. The faculty of the bacteria of fabricating poisons presented no interest to the bacteriologists, for it belonged to the common bacteria, those of putrefaction, which live on materials deprived of life, while the pathogenic bacteria were characterized by their power to develop in the living organism and to invade it.

We must remember that bacteriology originated

in the study of fermentation, with which naturally the infectious disease came to be assimilated. As alcoholic fermentation had found its provisional explanation in the life without air of the yeast plant, so the disease was explained by the life of the microbe in the animal body.

Thus, for example, in the deadly anthrax, whose study has been intimately associated with the beginnings of bacteriology, and which long served as the type of the bacterial diseases, the animals died with a great quantity of bacteria in the blood of all the organs. It was suppesed that the death of the animals was caused by this enormous mass of foreign bodies invading the animal humors, and the mechanism of the disease was explained by the mechanical effects of the presence of these foreign bodies in the blood, and the capillary emboli therefrom resulting. Writers also insisted on the evils resulting from the subtraction of oxygen from the red globules by the anthracoid bacteria, the lack of oxygen in the tissues, and the asphyxia therefrom resulting. They also had much to say about the asphyxia of the animal by the carbonic acid set free by the microbes.

In a more general way they directed our attention to the energetic nutrition of the bacteria, which get possession of the pabulum of the animal cells and kill them when starved.

Oftener still they contented themselves with vague expressions about the struggle for existence

between the animal cell and the microbe, without attempting further to analyze this vast conception.*

This was, we repeat, because the rising bacteriology was dominated by the analogy between the infectious disease and fermentation by figured bodies. Just as the decomposition of matters in fermentation was produced not by a chemical substance, or enzyme, but by the life without air of the ferments, so the infectious disease was the corollary of the life of the pathogenic bacteria in the animal body. And we have just seen that absorption of oxygen by the bacteria was especially invoked in explanation of the mechanism of the disease.

At the same time, the idea of intoxication by chemical poisons, which at an early period dominated infection, was not completely given up. Davaine believed that the anthracoid bacteria secrete a product which agglutinates the red globules of the blood. Pasteur, by the filtration of anthracoid cultures, proved the existence of this agglutinating diastase. Toussaint invoked the existence of a phlogistic poison produced by the anthracoid bacteria. He believed that he could even isolate it and confer with it immunity against charbon. Chauveau also adduced arguments in favor of this chemical vaccination by the bacterial poisons.

But chemical vaccination found a formidable op-

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*Duclaux: "Le Microbe et la Maladie." Paris, 1866.

ponent in Pasteur, who saw in it the overthrow of his vitalist doctrine of fermentation and disease. As in the fermentations the diastases or soluble ferments play only a secondary rôle in preparing the substances for the principal transformations effected by the living ferments, so the diastases of the pathogenic bacteria can only serve to explain the relatively unimportant symptoms of the disease which itself, as well as the chemical vaccine, was due solely to the life of the bacteria.

But in proportion as bacteriology made its new conquests, the insufficiency of this pathogeny of the infectious diseases became the more manifest.

In 1884, Löffler found the bacillus of diphtheria. He showed that this bacillus is always strictly localized in man to the mucous membranes which are the seat of the lesion. So in animals we only find it at the site of inoculation, and not in the internal organs, where it cannot live.

This microbe, which does not invade the organism, is nevertheless very pathogenic. Löffler showed that this pathogenic action is due to the production by the bacillus of a violent chemical poison, which is absorbed at the place where the bacillus makes its habitat and vegetates.

The problem of microbian poisons still more imperatively urged itself on the attention of the profession when Koch discovered his cholera-vibrio.

Koch demonstrated that the comma bacillus does

not penetrate the internal organs of the victims of cholera, but remains always limited to the intestinal canal. It, however, produces the grave symptoms of cholera poisoning. Koch concluded that the comma bacillus secretes a violent poison which explains the clinical phenomena of the disease. His prevision turns out to be supported by experiment. Cholera cultures sterilized in totality, or filtered of bacteria, and even the ptomaïnes extracted from these cultures, have been found endowed with a toxicity more or less appreciable.

A third bacterial disease was soon found to be absolutely inexplicable without the intervention of a soluble poison. This is tetanus. The bacillus of tetanus, discovered by Nicolaier, is strictly limited to the place of its inoculation. It nevertheless produces a terrible constitutional intoxication. Brieger sought for the tetanic poison, and found in the cultures of the bacillus and in the tetanized muscles several specific ptomaïnes which provoked convulsions in animals.

The great importance of the microbial poisons was emphatically demonstrated, thanks to the triumph of the doctrine of chemical vaccines.

The possibility of vaccinating animals against infection by the soluble products of bacteria, affirmed in the case of charbon by Toussaint and by Chauveau, and denied by Pasteur, was established several years later by the researches of numerous experimenters—

e.g., Wooldridge for charbon, Salmon and Smith for hog cholera, Beumer and Peiper for typhoid fever, Charrin for the pyocyanic disease. Latterly researches on chemical vaccinations have multiplied, and we may without exaggeration affirm that the acquisition of immunity in infectious diseases is a fact, thanks to the chemical products of the microbe. Lastly, we have succeeded in separating the microbes from their chemical poisons by filtration.

The decisive step in this direction was taken by Roux and Yersin. They found that after filtering through the Chamberland filter the cultures of the diphtheria bacillus, they obtained, with the filtered liquid entirely deprived of bacilli, toxic effects on the animals under experimentation. These toxic effects are obtained with minimum doses, and resemble the infection after incubation or after inoculation with the living bacilli. We may, for example, produce by the injection of filtered cultures the same diphtheritic paralyses as with the bacillus itself. This identity of effects constitutes a real demonstration of Löffler's notion that the diphtheritic bacilli act on the animal economy by the poison which they produce.

The researches of Roux and Yersin were soon confirmed by Löffler himself and by many other experimenters.

It was also found that diphtheria is not the only disease producible by the chemical products of the specific micro-organism. Filtered cultures of the

bacilli of tetanus were found by Knud-Faber, Tizzoni, Vincent, and others, to be extremely toxic. These filtration products, injected in animals, reproduced in them all the symptoms so characteristic of tetanus. Lastly, in the case of cholera also, I have succeeded in reproducing all the typical symptoms of the disease with the cultures of Koch's vibrio sterilized by discontinuous heat. From many other microbes, as we shall see as we go on, poisons more or less virulent have been separated.

At the onset of this period of the discovery of bacterial poisons, it was believed that we should have to distinguish two classes of infectious diseases. In one class would be ranged the infectant microbes which produce the disease and kill solely by their abundant development in the body of the animal; as types of this form, we were referred to charbon, tuberculosis, pneumonia, septicæmia in the hare. These microbes were supposed not to have the power to fabricate poisons. In the second class we had the toxine-making microbes, as those of cholera, tetanus, diphtheria, which cannot pullulate abundantly in the animal economy, but are endowed with extreme toxigenous power.

But this distinction is not legitimate. First, the same microbes produce in certain animal species general septic maladies, and local lesions in other species: the anthracoid bacterium and the lanceolate streptococcus of pneumonia are examples. A still more im-

portant argument against this distinction is that in the case of the so-called infectant microbes, we have succeeded in finding the poisons which they prepare. Thus the anthracoid poison has been described by Martin and Christmas; the pneumonic poison by G. and F. Klemperer; the tuberculous poisons by Koch, Maffuci, Prudden and Hoddenpyl, Strauss, and myself.

By all the researches which we have reported in this chapter, it has been established to a certainty that the pathogenic microbes act on the animal organism by means of the poisons which they produce. From this point of view the importance of the microbian poisons becomes extremely great.

At the outset of our historical survey we saw that systemic intoxication, to Hiller and his partisans, dominated infection; the poisons "formed in the midst of the tissues in decomposition" were everything, and the microbes nothing, in the disease.

Later the importance of these two factors was inverted: the intoxication was a common place phenomenon, meriting no special attention; the entire disease was produced by the progressive invasion of the living animal by the microbe.

To day intoxication has reconquered its rights. Infection is considered as an intoxication, but it is a special intoxication by the specific poison of a pathogenic bacterium.

Progress, as Hegel has defined it, traverses three

successive stages. The second is the negation of the first. The last approaches the first in its form, but explains the contradiction of the first two phases.

Second Part: General Microbial Toxicology.

CHAPTER V.

THE CHEMICAL NATURE OF THE BACTERIAL POISONS.

SUMMARY.—*Different Conceptions of the Chemical Nature of the Bacterial Poisons. First Stage: The Ptomaïnes—Differences between the Ptomaïnes of Selmi, of Gautier, and of Brieger—The Peptotoxine of Brieger—Researches of Salkowsky, of Bouveret and Devic—Researches of Bassi on Typhotoxine—Work of Baumann—The Ptomaïnes are Perhaps Artificial Products—Opinion of S. Martin. Second Stage: The Diastases—Researches of Roux and Yersin—Criticism of the Notion of Diastases. Third Stage: The Toxalbumins—Work of Brieger and Fränkel.*

The conceptions of the bacteriologists on the chemical nature of the bacterial poisons have already gone through several successive stages, under the influence principally of other toxicological researches of the same epoch.

Panum, whose brilliant achievements deserve so often to be cited, had found that the principal poison of putrid matters is insoluble in alcohol, and resembles certain albuminoid substances which, like the peptones, are not modified by boiling. But since Panum's researches, the attention of investigators has been directed more and more to toxic substances which are soluble in alcohol and are analogous to the most formidable of known poisons, the vegetal alkaloids.

Of the putrid poison of Gaspard and of Panum, Selmi, Gautier, and Brieger studied only one part, the ptomaïnes. Regarding their task from a purely chemical point of view, these three chemists bestowed but little study on the physiological action of the toxic substances they were testing. Nor did they compare, as Panum did, the toxicity of the primary poison with that of the different extracts obtained from it. The sole end of Selmi, Gautier, and Brieger was to extract from the putrid matters in a state of perfect purity certain definite chemical bodies, the ptomaïnes, whose pre-existence in these matters did not seem to them to merit a special demonstration. All three have succeeded in their efforts.

But it would be very instructive to enter a little more into detail in the examination of the results which they have obtained, and to compare the different ptomaïnes of these three writers.

Selmi found some cadaveric alkaloids extremely

like those of vegetal origin. Thus, for instance, he has isolated a cadaveric coniine, which by all its chemical reactions and by its physiological action is not distinguishable from the vegetal alkaloid.

The other chemists, contemporaries of Selmi, found also, as we have already seen, ptomaïnes remarkably like the veritable alkaloids. We need only mention the bases which resemble conicine (Sonnenschein), nicotine (Wolkenhaar), atropine (Tuelzer), veratrine (Brouardel and Bontmy).

Later, Gautier, Nencki, Quareschi, and Mosso found other ptomaïnes which constantly accompany putrefaction. These were collidine, parvoline, corindine—bodies less complex than the vegetal alkaloids, but still belonging to the pyridic series.

Later still, Brieger studied putrefaction under its various aspects. He discovered everywhere new ptomaïnes, but did not succeed in finding those of Selmi, nor even those of Gautier. The ptomaïnes of Brieger are diamines, and do not belong to the pyridic series.

To what can these constant differences be due, and why have these chemists constantly found these different products?

Evidently, we must hold in suspicion their different methods of extraction of ptomaïnes. We might even be warranted in believing that these methods were likely to create artificial products which did not pre-exist in the matters analyzed. We cannot here

enter into the chemical details as to the methods employed by Selmi, Gautier, and Brieger; we will content ourselves with the analysis of only one special point of this question. We will study the history of the peptotoxine of Brieger. This history has a capital importance in connection with the entire question of ptomaïnes and their origin in albuminoid matters.

We have already seen (see Chap. III) that Brieger found a toxic ptomaïne among the products of peptic digestion. In subjecting the different species of albumin, or even of peptone, to digestion with gastric juice, Brieger discovered a toxic body soluble in ethyl and amyl alcohol, giving the characteristic reaction of the alkaloids. Brieger gave the name of peptotoxine to this ptomaïne, which he supposed to be an aromatic amine. As all albuminoid matters in undergoing decomposition begin by being converted into peptones, it is quite natural that Brieger should find his peptotoxine at the onset of all the putrefactions which he studied. It disappeared after the first week. We see what great interest is attached to peptotoxine. This toxic ptomaïne is the necessary stage through which pass all the albumins in being liquefied by the soluble ferments of animal or bacterial origin. Being formed at the expense of the albumins by the relatively simple mechanism of diastasic fermentation, peptotoxine might well serve as a key to explain the appearance of ptomaïnes with the intervention of microbes.

Salkowsky, who has studied with much care this

question of the peptotoxine of Brieger, has never been able to find it in the products of gastric digestion. He supposed that Brieger's alkaloid was produced by the intervention of putrefaction during and with the aid of the manipulations.* The question has just received an unexpected solution by the researches of Bouveret and Devic.† These writers have demonstrated that peptotoxine is an artificial product which is formed at the expense of albuminoid matters by the combined action of hydrochloric acid and of alcohol.

Already Tanret had insisted on the close kinship between the peptones and the alkaloids. He had even shown that by the action of soda on the peptones, we may form alkaloids extractable by ether. Later, Dreschel saw that albuminoid substances boiled with acids yield among the products of their decomposition the organic bases. Bouveret and Devic have now shown that even less energetic reagents cause peptotoxine to appear at the expense of the proteids. The evaporation of albuminoid substances at 39° C. in presence of free HCl, leads to the formation of bodies which by the action of alcohol give rise to peptotoxine. There forms, by heating with a feeble excess of the free acid, an intermediate body at the expense of the soluble albumins. This intermediate body (santonine or acid albumin?) is decomposed by alcohol, and yields to the latter the toxic ptomaïne

* Salkowsky, Virchow's Archiv, 1891, t. 124, p 409.

† Revue de Méd., 1892, Nos. 1 and 2.

which did not exist before the action of acid and alcohol. These important researches throw a new light on the question of ptomaïnes.

The method of Brieger, employed in all his researches, begins ordinarily by the evaporation of the first matters in presence of hydrochloric acid, and by the extraction of the residue by alcohol. Now this initial operation already gives rise to the artificial production of ptomaïnes. It follows that the results obtained by Brieger can give us no information as to the poisons which pre-exist in the matters subjected to his investigation. They disclose to us only the products, more or less constant, of decomposition of these matters as a result of varied and complicated reactions. And even, sometimes, we do not know whence come these products: from the normal albumins or from microbian poisons? Thus, for instance, although Kulneff found, by Brieger's method, ethylendiamine in the contents of a dilated stomach, we believe with Bouveret and Devic that this body was introduced by the operations.

What must we think of numerous other ptomaïnes found everywhere by Brieger?

As for the typhotoxine of Brieger, Bassi has shown that it does not pre-exist in the cultures of the typhoid bacillus, but that it may be extracted from them by the method of Brieger.* As it has not been

* Bassi: La Tifotoxine di Brieger (Gaz. Chim. Italiana, 1889, t. 18, p. 521).

obtained by the culture of any other microbe, it is possible that it may be a product of decomposition of the typhoid poison.

In tetanus, Kitasato and Weyl have found by the method of Brieger the different ptomaïnes which he had indicated. It is possible that these ptomaïnes may be formed by the decomposition of the primary microbian poison, and it is certain that they are not the poison itself.*

More recently, Baumann has found a new method for the detection of diamines. By this method he has rediscovered in cystinuria many of Brieger's ptomaïnes, and has thus put beyond doubt their independent existence in the urine. But Baumann's method, which is based on the employment of chloride of benzoyle, is too harsh for the microbian poisons, which are, as we shall see farther on, extremely fragile.†

We see, then, that all these researches on the ptomaïnes cannot give us any idea as to the chemical nature of the microbian poisons.

First, they depend on methods which are too energetic, which may form artificial products even with the normal albumins—bodies relatively stable.

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* Kitasato and Weyl, Zeitschrift f. Hygiene. t. viii, p. 404.

† Udransky and Baumann: Ueber das Vorkommen von Diaminen, etc. (Zeitschrift f. Physiol. Chemie, 1889, t. 13, p. 562).

And one has no idea of the decompositions which they may effect in the microbian poisons.

Secondly, these researches have been made too exclusively from the point of view of pure chemistry; while the useful study of the microbe poisons is impossible without the constant control of experimentation. Naught but experimentation on animals can enlighten us as to the toxic effects proper to the poisons of the different microbes. This alone can indicate whether, after the different chemical manipulations, we have not destroyed or decomposed the primitive microbian poison, or substituted another for it. This true method of microbian toxicology has not been followed by Brieger and other seekers after ptomaïnes.

We may note here that Martin, in studying the poison of charbon,* comes to the same conclusion as ourselves; to wit, that the ptomaïne which one finds in the anthracoid cultures does not pre-exist there, but is there combined with an albuminoid substance.

On the other side, microbian toxicology has succeeded in showing, in accordance with Panum, that the toxic products of bacteria are not soluble in alcohol.

Arloing has extracted from cultures in bouillon of the *pneumococcus liquefaciens bovis* a toxic phlogogenous substance which is precipitable by alcohol,

* See also in this connection, Seams Woodhead, "Bacteria and their Products," London, 1891.

soluble in water and glycerin, and whose toxicity is destroyed by heating above 110° C. Arloing gave the name of diastase to this toxic substance, solely because among the microbian products only the ptomaïnes and diastases were known. The former are soluble in alcohol, the latter are not.*

Shortly afterward, Christmas also observed that the *staphylococcus aureus* produces in cultures a basic substance precipitable by alcohol.

Roux and Yersin experimented with the filtrate of diphtheria cultures (obtained through the Chamberland filter), and published several interesting reactions of the same. This poison is enfeebled or destroyed by heating above 60° C. It is not soluble in alcohol. It is carried down by the different precipitates which form in the midst of the liquid which contains it; *e. g.*, under the action of phosphate of lime, alumina, etc.

Roux and Yersin conclude that by all these reactions the diphtheritic poison resembles diastases; and none the less by its intense activity in infinitesimal doses.

This identification of the microbian poisons with the diastases was neither original nor new, for it had long been a habit of chemists to attribute to diastases every mysterious and unknown action exercised by a substance of indeterminate nature. Thus we have seen that Virchow, Schwenninger, and Stich affirmed

* Arloing, "Les Virus." Paris, 1891.

the diastasic nature of the putrid poison. The venoms of serpents have for a long time been regarded as soluble ferments. More recently the poison of jequirity was considered an enzyme. This notion has not, however, given any real advancement to science, for the diastases themselves are perfectly unknown to us in their chemical properties.

There is one exception, however: the diastases are characterized by their determinate chemical action on other substances. Pepsin, trypsin, and papaïn dissolve albuminoid substances; ptyalin and maltine break up starch into dextrine and glucose; invertin inverts cane sugar; emulsin and saponin decompose the glucosides. Now the only really scientific way of ranking the microbian poisons among the diastases would be to identify them from the point of view of this determinate chemical action. It is necessary to show that these poisons exercise a hydrating action on a certain class of substances, and that their toxic action proceeds precisely from this chemical reaction. No serious attempt has been made in this direction by the partisans of the diastasic nature of the microbian poisons. They have appealed, it is true, to the great activity of these poisons in very small doses. But from this point of view we should have to rank among the diastases, hydrocyanic acid, metilcarbylamine, the alkaloids such as nicotine, glucosides such as digitalin, salts of metals like corrosive sublimate, metalloids like phosphorus and fluorine.

But there are still serious arguments against the identification of the microbian poisons with the known diastases. Fermi and others have studied the different enzymes secreted by the microbes, and have found that they have no appreciable toxic action.*

It follows that the toxic action of the bacteria is not linked to their fermentative action; it does not coincide with any known diastasic action. As for any new diastasic action, we cannot deny it; at the same time we cannot affirm it till we have found it. It is evident that the toxic action of these poisons is reduced to some sort of chemical reaction, but we are far from knowing this reaction. Consequently, to say that the microbe poisons are diastases, is to speak inexactly if we mean any diastases actually known, and to juggle with words if we suppose a new diastase with unknown action. On the other hand, we cannot deny the numerous analogies between certain poisons and the soluble ferments—analogies which we shall indicate later on. We must come down to the researches of Brieger and Fränkel before we find the first serious attempt to determine the chemical nature of the microbian poisons. This attempt has had much success, although it was not made under the conditions one could have desired.†

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* Fermi: Die Hydrolytische Enzyme (Cent. f. Physiologie, 1891).

† Brieger and Fränkel: Untersuchungen über Bacteriengifte (Berlin. Klin. Woch., 1890, Nos. 11 and 12).

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Brieger and Fränkel first studied the diphtheritic poison. They found that this poison behaves with the different reagents like an albuminoid substance. This poison is precipitated by the neutral salts in excess, such as the sulphate of ammonia and sulphate of soda. It is not precipitated by sulphate of magnesia. It is precipitated by absolute alcohol, and is very soluble in water. By repeated precipitations by alcohol, and dialysis (contrarily to Roux and Yersin, Brieger and Fränkel have seen that the poison does not pass through the dialyzing membrane), the substance was obtained in a state of purity. This substance gave the following reactions: It is not precipitated by ebullition, sulphate of soda, common salt, sulphate of magnesia, dilute nitric acid (even with the aid of heat), or by acetate of lead. It is precipitated by carbonic acid (in saturated solutions), by the concentrated acids, by acetic acid and ferrocyanide of potassium, by phenol, by the organic acids (soluble in excess), by sulphate of copper, by nitrate of silver, by corrosive sublimate. It is lævogyrous. From all these, and other reactions not mentioned, the writers conclude that their toxic substance is exceedingly like serum-albumin. They have even analyzed this substance and found that it gives the elementary composition of the albumins.

Brieger and Fränkel have also studied, but more superficially, the other microbe poisons. They have found, beside the toxic albumins, certain globulines,

which are distinguished from albumins by their insolubility in distilled water.

CHAPTER VI.

THE CHEMICAL NATURE OF THE BACTERIAL POISONS—(Continued).

SUMMARY.—*Criticism of the Work of Brieger and Fränkel—Researches of Proskauer and Wassermann—The Microbe Poisons have Not yet been Prepared in a State of Purity—Similar Non-Microbian Poisons—The Work of Stillmarck—Demonstration of the Albuminoid Nature of the Diphtheritic Poison—Toxicological Differentiation of the Bacterial Poisons—Natural and Artificial Poisons: Their Characters—The Chemical Vaccines—The Antitoxines or Curative Substances—Hypothesis as to the Chemical Nature of the Poisons—The Nucleo-albumins and the Nucleines—The Consequences of this Hypothesis.*

We must dwell longer on the toxalbumins of Brieger and Fränkel, for the researches of these writers have captivated the scientific world by their apparent chemical rigorousness, and were followed by the creation of toxalbumins, toxalbumoses, and toxopeptones from every source. The conclusions of Brieger and Fränkel, nevertheless, call forth grave objections. And first, in order to prove that the diphtheritic bacilli produce a special albumin, have they eliminated the albuminoid substances from the bouillon?

Far from that, they have even added to the ordinary bouillon which serves for the cultures the serum of blood. How, then, can they believe that this serum-albumin which they find again at the end of their purifications is not the substance they introduced, carrying down in its precipitations, as Panum had already shown, the true microbian poison?

This supposition becomes a certainty when we regard the feeble toxicity of the pure substance of Brieger and Fränkel, compared with the far greater toxicity of Roux and Yersin's impure product of their calcic precipitate. In fact, Brieger and Fränkel have less right to say that the diphtheritic poison is a tox-albumin than one would have to affirm that it is phosphate of lime.

But phosphate of lime is a body well known, and it is very easy to tell whether it contains impurities. The albumins are much more difficult to analyze. The proof of this is that Brieger and Fränkel have not been able to show us isolated the albumins of broth and of serum, on the one hand, and the pretended toxic albumin on the other. There is no doubt, then, that in the experiments of Brieger and Fränkel the diphtheritic poison was always mixed with the albumins of the culture media, and that the chemical reactions described by them were those of the latter.

It may seem strange that these writers, when studying the chemical nature of the microbian poisons and finding them so closely associated with

albumins, had not thought to first separate the albumins from their cultures, leaving the microbes to vegetate in media deprived of albuminoid substances. But they had the preconceived idea that the bacterial poisons could only be formed at the expense of the albumins of the culture media. They give us no proof of this notion, so widespread and yet so inexact, as we shall show later on.

But even apart from the cultures in media deprived of albumins, there were many other means for determining the chemical nature of the bacterial poisons.

We might, for example, study what chemical changes coincide with the heating of the filtered cultures above 60° C.—a temperature which destroys the diphtheritic poison. We offer this suggestion because Brieger and Fränkel have made researches which may seem of a like nature. They have studied the non-toxic albumin produced by the emasculated diphtheria-bacillus, and found it distinguished by several constant characters from the albumin containing the diphtheria poison. First, it is brown, while the latter is white. It is soluble in dilute alcohol, and combines with phenilhydrosine, while the toxic albumin does not combine with that body, but with chloride of benzoyl. It contains more carbon, less oxygen and nitrogen, than the toxic albumin. As the virulent bacilli are distinguished from the attenuated by the energy of their assimilation, it is clear that this dis-

tinction between the different albumins of their cultures found by Brieger and Fränkel must be of complex origin, and does not permit us to judge as to the nature of the diphtheritic poison.

We must conclude that, despite all the chemical parade and apparatus of their work, Brieger and Fränkel have not succeeded in proving the legitimacy of their conclusions that the microbian poisons are albumins. Moreover, the same verdict has been reached by the authors of another work, emanating also from Koch's laboratory—Wassermann and Proskauer.

These writers have found in diphtheritic cultures the two albumins of Brieger and Fränkel—the yellow and the white. They have seen that the yellow albumin is especially preponderant in the cultures deprived of toxicity; it is not itself toxic. The white albumin was more abundant in the toxic cultures, and possesses a great degree of toxicity; by its chemical reactions this toxic albumin corresponds to what Kuhne calls *albumose;* it is precipitated by alcohol even when dilute, by the concentrated acids, the salts of the heavy metals, by potassium ferrocyanide, and by acetic acid. It is not precipitated by ebullition, by nitric acid, by basic acetate of lead, by sulphate and chloride of sodium in excess.

But Wassermann and Proskauer are far from being prepared to identify the diphtheritic poison with this albumose. They remark, on the contrary,

that the ordinary bouillon of culture always contains certain albumoses by reason of the infusion of meat. There does not exist, therefore, any serious argument to favor the view that the toxic body which they have isolated is not the same albumose of the bouillon with which is found mingled the poison. This last idea is confirmed by the fact that different portions of albumose isolated from the same toxic culture differ in their toxicity; they have, evidently, carried down varying quantities of the poison. The German writers conclude that the albuminoid nature of the diphtheria poison is possible, but that it has not been proved.

From all these researches we infer that the diphtheria poison is closely associated with the albuminoid substances, and more particularly with albumose, whose reactions it shares.

Just as in the case of the ptomaïnes, when chemists sought for substances analogous to the alkaloids of vegetal origin, the study of microbian toxalbumins clearly discloses a parallel tendency in the efforts and acquisitions of biological chemistry and of toxicology —*i. e.*, towards the toxic vegetal and animal proteids. In the seeds of different plants, in the poisons of serpents, in the blood and bodies of certain animals, were found certain substances of extraordinary virulence not belonging to the class of alkaloids. We cannot, unfortunately, enter here into the details respecting all these important researches. We will content ourselves by mentioning here the writings of

Warden and Waddel, of Martin and of Kobert, on abrine; those of Weir Mitchell and Edward Reichart and of Wolfenden on the poisons of serpents; those of Kobert on the venomous spiders; those of Mosso and of Kumahava on the poisonous fishes, etc.*

We shall here analyze only one of these memoirs —that of Stillmarck, composed under the direction of Kobert, on *Ricine*. Stillmarck has found that it is possible to extract from the seeds of the *ricinus* a substance extraordinarily toxic, which presents all the reactions of albuminoid bodies. It is not soluble in alcohol, in ether, or in distilled water. It dissolves readily in saline solutions. It is precipitated by ferrocyanide of potassium and acetic acid, by saturating solutions containing it with the neutral salts, and by ebullition.

Stillmarck supposes that this substance belongs to the class of albumoses of Kuhne. Unlike these, however, it is coagulated by heat.

As for the important question whether we can identify the poison with this albuminoid substance, or if the poison is simply mixed with the latter, Stillmarck advances serious arguments in support of the first alternative. First, by boiling, ricine is coagulated and rendered completely insoluble; at the same time, its toxicity is completely abolished. Then again, pan-

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* *Vide* the entire literature in Hulliburton's Text-Book of Chemical Physiology and Pathology. London, 1891.

creatic juice, which digests albuminoid substances, destroys also the toxicity of ricine.

It is rendered very probable by these researches, that there exist certain albuminoid bodies of nature, yet undefined, which are very violent poisons. We have recently shown that the diphtheria poison must also be ranked in this category, for it is destroyed, or at least modified, by the ferments of the albuminoid bodies.

Now to what class of albuminoid bodies do the microbian poisons belong? This question is the more important because these poisons have an evident kinship to a large class of vegetable and animal poisons of which we have before spoken.

Unfortunately, the data which biological chemistry possesses respecting the albuminoid matters in general are still very incomplete and indefinite. The methods for their analysis are still very imperfect. It is evident that under these conditions one cannot rigorously determine the chemical nature of the microbian poisons. We have already seen that even the best studied of all these poisons, that of diphtheria, has not yet been obtained in a state of purity, unmixed with the albumins of the culture media.

However, we possess for the microbian poisons a precious and powerful means of analysis, namely, experimentation, which is based on the property of these poisons to produce in animals certain phenomena similar to those of the infectious diseases: intoxication and immunity.

This means has enabled us to establish a fundamental distinction between two classes of microbian poisons: the natural or primary poisons, and the modified or artificial poisons. I have shown that in cholera there exist two poisons totally different in their physiological effects; the one produces the diarrhœa, while the other has a phlogistic action. These poisons differ also by their resistance to heat; the first is destroyed by heating it above 60° C., while the second supports a temperature of 120° C. for several hours. It is a tenable supposition that these two poisons are closely associated together, and that the second comes from the decomposition of the first.*

As for the diphtheritic poison, I have also found that it is necessary to distinguish the secondary poison which appears on the decomposition of the primary poison by heat, by acids, by the soluble ferments.

The attentive study of the other different poisons enables us to classify them also in one or the other of these two groups, which we shall characterize more in detail.

The natural poisons correspond to those which Brieger and Fränkel have called the toxalbumins, and Klemperer the toxines. They are substances which reproduce more or less exactly the symptoms of the

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* Gamaleïa: Experimental Researches on the Poisons of Cholera (Arch. de Méd. Experimentale, 1892, No. 2).

infectious disease. They are very unstable, and are decomposed by a temperature above 60° C. The animals which are refractory to the disease produced by their microbe, are also refractory to the primary poison of this microbe.

The modified poisons, which are also named *proteins* (Buchner, Klemperer), do not reproduce the typical phenomena of the microbian disease. They cause hypothermia or fever, according to the dose, inflammation more or less intense at the point of inoculation, and symptoms more or less pronounced of cachexia. They have the remarkable property of exciting tuberculous animals to general and local reaction. These poisons resist boiling. They are precipitated by alcohol. Panum's poison probably belongs to this class of substances. These poisons have nothing to do with the production of immunity. In certains cases, on the contrary, they have an opposite influence, predisposing the economy to the invasion of microbes. The vaccinated animals are not refractory to these artificial poisons. By the side of these primary and secondary poisons, experimental analysis has revealed still a third and a distinct order of substances. These are the chemical vaccines—the microbian products which confer on animals immunity against infection by the living microbe. In certain cases the chemical vaccination may be obtained by means of the primary poison—as, for instance, in the vaccinations against the septic vibrio,

or against the streptococcus of pneumonia. But in many other cases the primary poisons do not confer immunity, but lead, even in small doses, to cachexia. Such is the case with regard to tetanus and diphtheria. But here also a chemical vaccination is possible by means of certain artifices. Thus, for instance, we prepare the vaccines by heating the primary poisons above their critical point, to 70° or 80° C. We may suppose that this heating sets free the vaccinal substance of the primary poison. This method succeeds for pneumonia, and sometimes for tetanus and diphtheria. In some diseases chemical vaccination is easier; the microbian products vaccinate, even after having been subjected to a temperature of 100° and even 120° C. This is the case with cholera and the vibrionic septicæmia, which I was the first to discover.

In certain cases the vaccinant products have been quite isolated from the primary or modified poisons. I have found that against cholera and against the avicide vibrio we may vaccinate with the volatile products of the cultures. As for the proteus vulgaris, it has been seen that we can vaccinate by the different ptomaïnes, such as neuridine, muscarine, etc. Thus we see that the chemical vaccines are very varied. We may say, in general, that they are more stable than the corresponding poisons. I have regarded them as a decomposition-product of the poisons; in any event, they have a variable chemical nature, being decomposed by ebullition.

There exist, lastly, substances still more complex, which are found only in the organism of refractory animals after an injection of the poisons. These substances, which are combinations of poisons with the animal substances, or possibly chemical vaccines quite pure, have the property not only of vaccinating the animals against an infection to come, but of curing the disease when declared.* The nature of these immunizing and curative substances is completely unknown.

If we wish now to sum up all this long study of the chemical nature of the microbian poisons, we arrive at the following conclusions:

Among the products of the microbes, there are four classes of substances: *First*, the organic bases (which, if they do not exist essentially as ptomaïnes, assume that form under the different processes of analysis); *second*, certain substances of albuminoid nature, or primitive poisons, easily decomposible at a temperature above 60° C.; *third*, still other albuminoid substances, which are more stable—the modified poisons; *fourth*, a class of substances which can only be defined by their physiological action, the chemical vaccines—these are associated with one of the three preceding classes of microbian products.

Despite all this diversity of the microbian products, and all the apparent complexity of their relations,

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*Gamaleïa: Immunisation (Gaz. Hebd., 1891, No. 47).

we may frame a hypothesis to explain their chemical nature.

Biological chemistry has taught us that there is a particular class of substances more complicated than the ordinary albuminoid substances, extraordinarily unstable, decomposing even by prolonged contact with alcohol and by heat above 60° C., giving rise by their decomposition to the formation of other albuminoid substances more stable, as well as to the different ptomaïnes and leucomaïnes. These are the nucleo-albumins or vitellines, constituent parts of all animal and vegetable cells. I have made the hypothesis that the natural poisons of the bacteria are precisely such nucleo-albumins, which give rise to nucleines or modified poisons by their decomposition, and to ptomaïnes by a more profound decomposition. The principal chemical character of the nucleo-albumins is the great quantity of phosphorus which they contain. In the microbian poisons, chemists have already found much phosphorus. Such is the case in regard to the poisons of cholera (Petri), the avicide vibrio (Wolkow), and Koch's tuberculin. But all these acquisitions of organic chemistry have no decisive value, for no one of these poisons has been obtained in a state of purity—all have been mixed with other substances derived from the culture media. Till the determination of phosphorus shall have been made for a perfectly pure microbian poison, our idea will remain only an hypothesis. But it may be verified

upon other characters of the nucleo-albumins—characters which are less decisive from a chemical point of view, but much more interesting from the standpoint of microbian toxicology.

And, first, there arises the fundamental question: Are the microbian poisons the products of decomposition of the nutritive media of the bacteria, as current opinion has it, or are they the constituent parts of the bodies of the microbes, as they ought to be if they were nucleo-albumins? This important question concerning the origin of the microbian poisons will be examined in the next chapter.

CHAPTER VII.

THE ORIGIN OF THE MICROBIAN POISONS.

SUMMARY.—*Preconceived Idea that the Poisons Can Only Come from the Decomposition of Albuminoid Bodies—Experiments Already Old which Overthrow this Idea—Researches of Polotebnoff, Popoff, Bergmann, Schuller—Numerous Researches Made at the Laboratory of Pachoutine—Recent Labor of Guinochet—The Microbian Poisons Are Not Products of Decomposition, but the Result of Synthesis—Are They Secretions?—Arguments in Favor of this Idea—Arguments which Combat It—The Poisons are Intimately Linked to the Bodies of the Bacteria—The Experiments of Cantani and of the Author—The Researches of Buchner on the Proteins—Criticism of these Researches—All the Microbe Poisons Come from the Bodies of Bacteria—Utility of this Source for the Microbes.*

In all the history of the researches on the microbian poisons, the idea was generally admitted that these poisons take their origin in the course of the decomposition of albuminoid matters. This idea is prevalent at the present time. Nevertheless, there exist in science many facts which completely over-

throw it. As early as the epoch of the study of the putrid poison, chemists were seeking to eliminate the albuminoid substances from the matters undergoing putrefaction, in order to render easier the extraction of the putrid poison. Thus, for instance, Bergmann and Schmiedeberg had employed brewer's yeast in their famous experiments on sepsine.

Polotebnoff was the first to employ an entirely mineral medium and allow it to putrefy. It was Pasteur's liquid, composed of ammonia and of different neutral salts. Polotebnoff found that the liquid when putrefied is not more toxic than the fresh liquid.—Popoff, who repeated the experiments of Polotebnoff, has shown that these conclusions are not exact, and that Pasteur's liquid when putrefied produces absolutely the same symptoms of intoxication as the putrefied albuminoid matters. But Popoff did not kill the microbes in his liquid, and he supposed that it was precisely the microbes which provoked this septic intoxication.—The same experiments were made by Hugo von Brehm.—Bergmann has given to the question a decisive advancement. He has shown that Pasteur's liquid when putrefied preserves all its septic action, even after having been boiled.—Anders has also found that neither boiling nor filtration through clay destroys the septic action of this liquid.

Thus was established this principle of the highest importance: That the septic poison is the result of

the synthetical action of the microbes, and not of the decomposition of the putrid matters.

The same results were obtained by Schuller, who operated with the liquid of F. Cohn, which contains no sugar, and whose sole organic compound is ammonium tartrate.

These experiments have since been often repeated with the same positive results. These researches have been chiefly made at the laboratory of Pachoutine. We must mention especially the theses of Bottcharoff and of Cosorotoff emanating from this laboratory. All these writers, as well as numerous others who have studied the details of putrid intoxication—as, for instance, the fever and the gaseous exchanges—have uniformly arrived at this result: that the putrid poison is formed in the absence of every albuminoid body, and that it is, consequently, the result of the synthetic activity of the microbes. Note also that these writers have confirmed in general the datum of Panum as to the great toxicity of the products precipitated by alcohol. Nevertheless, contrarily to Panum, the alcoholic extract had often itself also a certain septic activity.

Unfortunately, all these experimental researches were carried on, not with pure cultures of bacteria, but with cultures containing the microbes of putrefaction in indefinite quantity. They were carried on from the point of view of experimental pathology, and bacteriology, which has mostly confined itself to

pure cultures of microbes, has rather ignored the important results established by these labors.

Of much more importance are the researches of Guinochet on the diphtheritic poison. Guinochet has found that the diphtheritic bacillus, which is easily cultivated in normal filtered human urine containing not a trace of albumin, produces its poison in that medium quite as energetically as in meat broth.*

Thus we find that there is little ground for the preconceived idea, which has lasted so long, that the pathogenic microbes fabricate their poisons by a special decomposition of albuminoid matters. This notion, demonstrated false, must give place to the more just conception, viz., that the poisons are the synthetic products of the microbes.

Independently of the theoretical importance of this conclusion of Guinochet, we must also indicate its practical side. We shall be able henceforth to study the formation of the microbian poisons in conditions more simple than those which were chosen by Brieger and Fränkel and their successors, in a medium totally deprived of albumins.†

The question now presents itself: What is the rôle of these substances? What place do they oc-

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*Guinochet: Labors of the Laboratory of Straus, at the École de Médecine, Paris, France.

†It only remains to find a culture medium favorable to the development of the microbes, for the mineral media (liquids of Pasteur, Nœgeli, and Cohn) are not suitable for

cupy in the life of the microbes? For the definition of animal poisons depends for its significance upon the relation of these poisons to the higher organisms, and not to microbe-biology.

There were already a certain number of synthetic and specific products known to microbiology, whose signification was sufficiently clear. These were the soluble ferments, or diastases, which the microbes secrete to render assimilable the aliments which surround them. The yeast-plant secretes invertine, which decomposes cane sugar, not directly utilizable, into dextrine and levulose. The bacillus of lactic acid secretes the lab-ferment (which serves to coagulate the casein of milk), and casease (which dissolves and peptonizes it).

It was natural to think of the similarity of these microbian manifestations to the empoisonments produced by the pathogenic microbes. One might readily believe that the poisonous secretions serve also to prepare the animal soil for the microbian life, whether by rendering the animal humors assimilable for the microbes, or by abolishing the vital resistance of the

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the pathogenic bacteria. I have found that they grow well in the following medium:

Water	1000
Liebig's extract	5
Glycerin	40
Common salt	5

Liebig's extract is totally exempt from albuminoid matters.

animals and thus rendering the field clear for the occupancy of the microbes.

This notion soon found a certain support in experimentation. It has been observed that the diphtheritic and tetanus poisons behave towards the different reagents precisely like the soluble ferments. It has also been noted that different substances—the soluble ferments of papaïn; such microbian products as the poison of the bacillus prodigiosus; the poisons of plants, like abrine—also possess the property of rendering the animals in which we inject them in minimum doses predisposed to the pullulation of microbes which are not pathogenic to the same animals in the normal state.*

Nevertheless this comparison of the poisons to the secretions accounts but superficially for the facts.

First, there exist certain pathogenic bacteria which produce such diseases (of the highest importance) as tuberculosis, cholera, and charbon, where we do not find any toxic secretions.

Then again, even the production of poisons in diphtheria and tetanus has a very superficial correspondence to a secretion. In fact, the quantity of these poisons in the culture liquids does not grow proportionately to the development of the bacteria themselves. On the contrary, an attentive observation reveals the following facts:

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*See Gamaleïa "On the Reproduction of Cholera in the Hare" (Congress of Berlin, 1890).

At the very first, and at the most active moment of the life of the microbes, the liquid of culture is acid and devoid of all toxic power. Later, when the bacteria have ceased to multiply and are deposited at the bottom of the culture-flask, the liquid becomes alkaline and more and more toxic. Its toxicity augments, in a certain measure, progressively with the duration of the sojourn of the bacteria in their alkaline liquid of culture.

The explanation of this fact is very simple. The poison is contained inside the bodies of the bacteria, and is extracted but slowly by the alkaline liquid in which they are macerated.

This hypothesis as to the origin of all the microbe poisons from the bodies of the bacteria has been already proposed by Cantani *à propos* of the cholera poison. Cantani supposed that in the cholera cultures it was the bacilli themselves which were toxic, like certain fungi which cause poisoning when injected. He did not prove his hypothesis, which long remained wlthout support. This was six years ago, and Cantani's article "*Die Giftigkeit der Cholera-bacillen*" was published in the Deutsche Medicinische Wochenschrift in 1886. Since then I have shown that the cadavers of divers bacteria remain very toxic even after having been exposed to a heat of 100° to 120° C.

Later, I showed that we can prepare from the cadavers of the cholera vibrio and of the avicide vibrio

very toxic extracts which possess at the same same vaccinal properties.*

At the same time that this study of the general action of the bacterial cadavers was being pursued, other experimenters were investigating their local effects. After Gravitz and Wyssokowitch had shown that the cadavers of certain bacteria have the property of producing suppuration in animals, Buchner made of this question a special study. By boiling with dilute alkalies, he succeeded in extracting from the bodies of different bacteria, such as the typhoid and the pyocyanic bacillus, substances in general of little toxicity, but possessing the property of producing by subcutaneous injection an exudative inflammation. With doses still more feeble, these poisons provoke only an emigration of leucocytes (positive chimiotaxy).

By their chemical properties these substances correspond quite well to those which Rencki had described under the names of *myco-protein* and *antraco-protein*. Buchner considered them as alkali-albumins or proteins, and under that name they have since been studied. Their most curious property has been set forth by Koch. Already in 1889 I had indicated that tuberculous guinea-pigs have an extreme susceptibility towards the vibrio poison. Koch has suc-

* Gamaleïa, in Ann. de l' Instit. Pasteur, 1888, No. 5; also 1889, No. 10; also in Compt. Rend. de la Soc. de Biologie, Nov. 30, 1890.

ceeded in extracting from the tubercle bacilli, by heating with glycerin, a substance called tuberculin, which proves to be extremely active in tuberculous subjects. It provokes in them the phenomena, so often described, of general and local reaction. This reaction of the tuberculous to tuberculin has long been wrongly considered as a characteristic effect of the latter. But latterly it has been shown that, besides the avicide vibrio and the bacillus tuberculosus, many other bacteria contain a substance having a similar property. Buchner and Röhmer have given the following directions for the extraction of these substances: Dry the bacteria after separating them from their culture media; boil them several hours in alkaline water, and leave the decoction to macerate for several days at the temperature of 37° C.; then filter the liquid, and precipitate the active substance by acetic acid.—Thus we see that, with the proteins of Buchner, the poisons extracted from the bodies of bacteria have acquired the right of place in bacteriology. But we must make some important reservations in regard to this conception of the proteins.

First, Buchner and the chemists who followed him established a fundamental distinction between the proteins and the toxalbumins or toxines—for the latter come from the secretions of the bacteria, the former only from their bodies. The proteins exercise a positive chimiotaxy, the toxines negative chimio-

taxy. (We shall return shortly to the rôle of microbian poisons in inflammation.) As to the source of these poisons, we have already seen that there are reasons to suppose that the toxalbumins are also contained in the bacteria. If Buchner has not succeeded in extracting from the bodies of microbes aught but the proteins, this is evidently due to his crude method of decoction, which surely destroys the toxalbumins. We have shown that in cholera one may obtain by extraction at a temperature of 55° to 60° C. another poison than that which is extracted by ebullition. Likewise, in respect to tuberculosis, if instead of Koch's process you employ Weyl's more efficacious method of extraction by alkaline water at low temperatures, you obtain a poison more active than tuberculin, and endowed with other physiological and chemical properties. Now it is more simple to suppose that all the poisons of the microbes come from their bodies; but that the extraction at low temperatures gives us the toxalbumins or primary poisons, and that ebullition gives the proteins or modified poisons.

Moreover, in coming back to the point of departure of the researches of Buchner—to the suppuration produced by the microbian cadavers—it is proper to note that this savant has completely failed in his attempt to find the pyogenic substances. The proteins which he has isolated produce an exudative inflammation, and not suppuration. Buchner has found the likeness of suppuration in the emigration

of the leucocytes, in the positive chimiotaxy provoked by his proteins. But this positive chimiotaxy not only has not the significance of suppuration, but is not even inflammation. It is nothing but hyperæmia. We see it when we introduce very small doses of proteins (in capillary tubes) under the skin of animals. When we augment the doses injected, we observe (as we have seen) the production of a serous exudative inflammation without leucocytes. So our positive chimiotaxy becomes, by the augmentation of the doses, negative chimiotaxy, and cannot serve to distinguish the two classes of poisons. But suppuration is not obtained with the proteins.

It is very probable that the failure of Buchner to extract pyogenic substances from the microbian bodies was due to his faulty process of extraction, and that the pyogenic substances belong to the category of the more fragile (the primary) poisons. As to the chemical nature of the substances extracted by Buchner from the bodies of the bacilli, their identity with the class of alkali-albumins or proteins cannot longer be admitted. Buchner had found that these substances present the reaction of albuminoids, and that they are, besides, precipitated by neutralizing with acetic acid their alkaline solutions. But this latter reaction is an artificial one, produced by reason of their extraction by alkalies from the bodies of bacilli. When Buchner later employed for this extraction neutral water, he no longer observed precipitation by the

acids. If we still continue to call these substances alkali-albumins, it is by an abuse of language. Likewise, Koch's tuberculin, which belongs by all its physiological properties to the same class as Buchner's proteins, but which is extracted by means of a neutral solvent, does not present the characteristic reactions of the alkali-albumins. It is evident, consequently, that the substances of Buchner, or our modified poisons, are not alkali-albumins. We have already stated in the preceding chapter that we believe them to be nucleines. As for tuberculin especially, our hypothesis is found confirmed by the richness of this substance in phosphorus, which we find even after having freed from phosphoric salts the bouillon in which the bacillus tuberculosis has vegetated.

Thus the modern researches on the origin of microbian poisons lead us to the same results as the study of their chemical properties. The microbian poisons may be divided into primary and secondary. They are the results of the creative synthesis of the bacteria, and come from their bodies. Thus is again confirmed our hypothesis respecting their chemical nature which identifies them with the constituent parts of the nuclei of cells—the vitellines and the nucleines.

The toxicity of the microbian bodies may have also a teleological signification, explicable from the point of view of the struggle for existence and of natural selection; for the toxicity of the bodies of

bacteria may be useful to them as a means of preserving them against aggressors which would devour them.

CHAPTER VIII.

ACTION OF THE BACTERIAL POISONS ON THE ANIMAL ORGANISM—ACCUSTOMANCE AND IMMUNITY.

SUMMARY.—*Insufficiency of our Knowledge of the Mode of Action of the Bacterial Poisons on the Animal Organism—Local Action of the Modified Poisons—Selective Action of Tuberculin and Malleine—Destruction of Poisons in the Bodies of Refractory Animals—Explanations of the Different Contradictions—Résumé and Conclusions on the General Toxicity of Microbes.*

As the microbian poisons have not been the object of systematic researches in their aggregate, certain important questions concerning their history are still undecided. The mode of action of the poisons on the organism is one of these questions. Even in the case of the best known of the microbian poisons, we do not yet know on what elements they act preferably, whether on the nerve-cells or muscles, on the heart or capillary vessels, or even on the blood.

Most of these poisons produce a local lesion at the place of injection. This local lesion may go on from a serous or sero-sanguinolent exudation to necrosis. This phlogistic action belongs to the

majority of the modified poisons. It is, too, a little singular that when injected into the blood, these same poisons have a marked antiphlogistic action. The intravenous injection of these poisons prevents the appearance of inflammation, even when solicited by very energetic agents, such as the application of croton oil. Bouchard has endeavored to explain this antiphlogistic action by the paralysis of the vasomotor centres; but this explanation is not fully demonstrated, and still remains contested (Samuel).*

When injected under the skin, but in very small doses, the same modified poisons provoke inflammation, not at the place of their introduction, but around foci of pre-existent lesions. The best known examples of this elective action are the local reactions provoked by tuberculin† in the tuberculous, and by malleine‡ in horses affected with glanders.

The attempt has seriously been made to explain this elective inflammation by the positive chimiotaxy

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*Charrin and Gamaleïa: On Inflammation (C. R. de la Société de Biologie, July 5, 1890). Gamaleïa: On the Local Lesion in Microbian Diseases (Arch. de Méd. Exper., 1891, No. 2).

†Koch, Deutsche Medicinische Wochen., 1890, No. 46. Gamaleïa: On the Treatment of Tuberculosis by Koch's Method (Arch. de Méd. Experimentale, 1891, No. 2).

‡Helmann: On the Clinical and Experimental Diagnosis of Glanders (Messager de la Science Vetérinaire Publique, 1891, No. 4).

excited by tuberculin when freed from glycerin. This explanation is at the best a strange one.

How can the positive chimiotactic power of tuberculin, *i.e.*, the action which it may exercise in certain conditions on the leucocytes, explain the exudative inflammation produced by tuberculin, not at the spot of inoculation, but at the place of election? This impossible comparison can only be understood by calling to mind the confusion which exists in certain microscopists with regard to the difference between inflammation and the emigration of leucocytes, the latter being only an element more or less constant of inflammation, and indeed oftener found apart from it.*

The explanation of the elective action of tuberculin is not, however, so difficult, and it has already been sketched by Koch. The acute inflammation is in general the consequence of a lesion or rapid destruction of the cells and tissues of the animal. When injected in sufficient concentration, the modified poisons induce cellular lesions and exudative inflammation at the place of their injection. Appropriately diluted, they are inoffensive to the normal cells of the economy, and affect only the tissues already diseased, around which is then produced the inflammatory process. This explanation agrees well with the

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*This confusion has even been made the basis of a new theory of inflammation.

property of other substances besides tuberculin to provoke reaction in the tuberculous, and with the property of tuberculin of provoking also reaction around certain lesions not tuberculous.

The relations between the microbian poisons and the animal economy have been chiefly studied from the point of view of immunity. When vaccination by means of the soluble products of bacteria was first demonstrated, the very natural idea arose that this immunity was acquired by the habituation of the animals to the poison. According to the different hypotheses respecting immunity, this habituation was supposed to affect either the entire animal economy, or only certain of its cells. This idea was, moreover, confirmed by the facts connected with one of the first chemical vaccinations known. Beumer noted that he could easily habituate mice to progressively increasing doses of the sterilized cultures of the typhoid bacillus. These mice, having become accustomed in this way to the poison, were able to sustain without injury the inoculation of large quantities of the living bacilli —a procedure which proved speedily fatal to mice not vaccinated.* Similar facts have been noted by Foa and Bonome in the chemical vaccination of hares against the proteus vulgaris.†

*Beumer: Der ḍerzeitige Shandpunkt der Schutzimpfungen, pp. 4–6. Wiesbaden, 1887.

†Foa and Bonome: Ueber Schutzimpfungen (Zeitschrift f. Hygiene, t. v, p. 415).

But in my own researches on vaccination against the avicide vibrio found by me, I have met with facts which overthrow this theory of habituation. The cultures of the avicide vibrio, sterilized at 120° C. and very toxic for guinea-pigs, easily vaccinate them against infection by the living vibrio. But after being once vaccinated the guinea-pigs do not become more resistant to the toxic action of the vaccine—*i. e.*, to massive doses—than the non-vaccinated. The fever which appeared after the injection of small doses was in them identical with that of the control-animals. The hypothermia following larger doses had the same characters. The exudative inflammation which the vaccine provokes at the point of inoculation was not modified in consequence of the vaccination of the guinea pigs. The phenomena of positive or negative chimiotaxy depended on the dose of vaccine introduced, but not on the immunity or non-immunity of the guinea-pigs. In short, the behavior of the guinea-pigs toward the poison of the chemical vaccine was in no way changed by the vaccination. Moreover, we have found that the volatile products of the non-toxic cultures can also vaccinate. We have found this same failure to undergo habituation in animals subjected to cholera vaccine,* to pyocyanic vac-

*The facts which we have found have been confirmed by many observers. See especially Hernandez, "Chemical Vaccinations," in C. R. de la Soc. de Biologie, July 4, 1891;

cination (Charrin and I), to hog-cholera vaccine (Selander).†

All these facts show that the chemical vaccination of animals does not depend on their becoming accustomed to the vaccine. They may be explained by the hypothesis that in our vaccine are mingled the poison, and the vaccinant substance distinct from the poison. This hypothesis of the distinction between the toxic substance and the vaccinant substance, formulated first by Bouchard,‡ may account for the mechanism of vaccination, but it is manifestly insufficient, for it does not explain the positive cases of resistance to the poisons by the vaccinated animals in the experiments of Beumer, Foa, and Bonome, and others of whom we shall speak presently.

The complete explanation of this difficulty can only be obtained by the aid of the notion of the plurality of the microbian poisons. We have established the distinction between the natural or primary poisons of the microbes, and their artificial or modi-

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and Metschnikoff and Roudenko, "Researches on Habituation to the Microbian Products" (Ann. de l'Instit. Pasteur, 1891, No. 9).

† Gamaleïa: On Cholera Vaccination (C. R. de la Soc. de Biologie, Nov. 30, 1891). Charrin and Gamaleïa: Vaccination et Accoutumance (C. R. de la Soc. de Biologie, July 5, 1890). Selander: Study of Hog-Cholera (Ann. de l'Inst. Pasteur, 1890, p. 545).

‡ Bouchard: Therapeutique des Malades Infectieuses, p. 137. Paris, 1889.

fied poisons.* With this distinction, all the foregoing contradictions are cleared up. If our animals vaccinated against the infection did not become refractory to the poisons contained in the chemical vaccines, this was owing to the fact that the latter poisons are artificial products and have nothing to do with the infection and the natural poisons of the bacteria.

In fact, towards these natural poisons the vaccinated animals become quite as refractory as towards the microbes themselves. This last fact was first established by the researches of Behring and Kitasato in reference to tetanus and diphtheria.† G. and F. Klemperer‡ have noticed the same resistance to the pneumonic poison on the part of animals vaccinated against the *streptococcus lanceolatus* of pneumonia. In cholera, lastly, we have seen that vaccinated dogs become refractory to the natural poison of cholera and not to the modified poison. Quite recently, also,

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*Gamaleïa: On the Diarrhœic Action of the Cultures of Cholera (C. R. de l'Acad. des Sciences, March 24, 1890). Gamaleïa: On the Two Poisons of Cholera (Bulletin Médical, April, 1890).

†Behring and Kitasato: Facts as to Immunity from Tetanus and Diphtheria (Deutsche Medic. Wochensch., 1890, No. 49).

‡G. and F. Klemperer: Immunity against Pneumococcus Infection (Berlin. Klin. Wochensch., 1891, Nos. 34 and 35).

in respect to typhoid fever, the researches of Beumer and Peiper on the resistance of vaccinated animals to the poison have been confirmed by those of Brieger, Kitasato, and Wassermann.*

We may consequently regard it as a very general fact that animals refractory to infection by a microbe also resist intoxication by the natural poison of this microbe. This principle finds verification, not only in reference to artificial immunity conferred by vaccination, but also to natural immunity. Thus, for instance, rats which are refractory to the diphtheritic infection, support without difficulty, according to Roux and Yersin, the injection of enormous doses of the poison of diphtheria.

We may conclude that immunity is linked to the resistance of animals to the microbian poisons.

To what is this resistance due? We have already seen that it is not the result of accustomance, for by a chemical vaccine which does not contain any natural poison we vaccinate against a natural poison.

Behring and Kitasato have shown that in respect to tetanus and diphtheria, acquired immunity and resistance to the poison are linked to the power of the serum of the vaccinated animals to destroy even *in vitro* the corresponding poison. This remarkable discovery has been since then often confirmed in its ap-

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*Brieger, Kitasato, and Wassermann, Zeitschrift f. Hygiene, t. xii, p. 137.

plication to diphtheria and tetanus, and also to other poisons.*

At the same time, this antitoxic property of the serum cannot be the cause of the resistance of animals to the microbian poisons; it is rather the consequence, for animals naturally refractory do not ordinarily possess this antitoxic property of the serum—they do not acquire it until after having destroyed the poisons in their bodies.† The immunity cannot therefore depend on the antitoxic power of the serum; it can only be characterized by the possibility of acquiring this antitoxic power after the introduction of the poison.

But the question as to the nature of immunity is foreign to our book, and we cannot enter into explanations respecting its physiological mechanism.

To sum up, briefly, all that we know as to the general toxicology of the microbes, we shall see that our hypothesis as to the chemical nature of the bacterial poisons, in absence of a direct demonstration which can only be made with the poisons isolated in a state of purity, is found confirmed by many indirect proofs.

That the microbian poisons belong to the class of nucleo-albumins, is in agreement with their extreme

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* Ehrlich: Experimentelle Untersuchungen über Immunität (Deutsche Medic. Woch., 1891, Nos. 32 and 44).

† Gamaleïa: L'immunisation (Gaz. Hebdom. de Méd. et de Chir., Nov. 21, 1891).

fragility, and the facility with which they give rise to the modified poisons on the one hand, to the ptomaïnes on the other. This idea is also confirmed by what we know about the source of the poisons of the microbian bodies, which are of nuclear origin. It is, lastly, compatible with the plurality of poisons associated together and produced by the same bacteria—a plurality postulated by the physiological study of these poisons.

We shall speak only of the principal facts which confirm our hypothesis.

Third Part: The Special Toxicology of the Microbes.

CHAPTER IX.

THE POISONS OF TETANUS.

SUMMARY.—*Researches of Brieger, Kitasato and Weyl, Knud Faber, Tizzoni and Cattani, Brieger and Fränkel, Vaillard and Vincent and Kitasato, on the Properties of the Tetanus Poison—The Investigations of Bruschetini and of Camara Pestana on its Diffusion in the Animal Body—Vaccination against Tetanus: Behring and Kitasato—The New Notions Contributed by these Authors.*

We begin our exposition by the study of the poisons produced by the bacillus of tetanus. These poisons are not the first known, but they are in many respects the best studied, of the microbian poisons.

Brieger was the first to investigate the toxic products formed by the bacillus of tetanus. In the cultures of this bacillus, as well as in the amputated arm of a patient, he found several characteristic ptomaïnes. There was tetanin, which has the property of provoking in mice, even in very small doses,

attacks of trismus and of tetanus ending in death; tetanotoxin, which provokes convulsive paroxyms, followed by complete paralysis; spasmotoxin, which causes cramps; and lastly, toxin, which has the property of stimulating the salivary and lachrymal secretion.

The researches of Brieger date from a period when we did not know how to cultivate the tetanic bacillus in a state of purity. Since then, Kitasato has succeeded in making pure cultures of tetanus. One may ask if in the pure cultures the ptomaïnes of Brieger may be found? Kitasato and Weyl have put to themselves this question. They have succeeded, by employing Brieger's method, in finding in notable quantity tetanin and traces of tetanotoxin. Among the volatile products of tetanus cultures they have found H_2S, butyric acid, indol, and phenol. But the two bases found were little toxic, and quite insufficient to explain the disease. Tetanin does not provoke convulsions and salivation in mice except when given in very considerable doses, and spasmotoxin causes only paralysis.*

It was evident (and this is the conclusion of these authors) that the true tetanus-poison must be found elsewhere.

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* Brieger: Ueber Ptomaïne, iii, Theil. Berlin, 1886. Kitasato and Weyl: Zur Kentniss der Anæroben (Zeitschrift f. Hygiene, t. viii, p. 404).

Moreover, already in 1890 Faber had found in tetanus cultures a poison of quite another nature than the ptomaïnes of Brieger. In filtering cultures of the tetanus bacillus through the Chamberland filter, Faber obtained liquids deprived of bacilli, but reproducing in animals all the symptoms of tetanus in the same degree as the tetanus bacilli themselves. Intoxication by the poison resembled infection by the bacillus itself in the two following respects: (1) both demand a certain time for their manifestation—a period of incubation for the intoxication as for the infection; (2) when introduced into the stomach, the chemical poison is quite as inoffensive as the living bacillus. Faber also observed that the toxicity of the filtered cultures was completely destroyed by a five-minutes heating to 65° C., and even by the addition of alcohol.

He concludes that the tetanus poison is not a ptomaïne, but rather a toxic diastase like that of jequirity or that of diphtheria. Faber had not at his disposal pure cultures of tetanus.*

But these results were soon confirmed by Tizzoni and Cattani. These authors have found that the tetanus bacillus produces its poison in pure cultures on gelatin, and not in such as are made in bouillon. The toxic substance of the filtered cultures is modi-

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* Knud Faber: On the Pathogeny of Tetanus (Berlin. Klin. Woch., 1890, No. 31).

fied by precipitation by alcohol. It does not dialyze, and this has enabled the authors to isolate it in the following way: Filtered cultures are supersaturated by ammonium sulphate in excess; a precipitate is formed, which contains the toxic substance; this precipitate is dissolved in water, freed from salts by dialysis, and evaporated in a vacuum at a low temperature. The toxic substance obtained in this way presents itself as a yellow body, of crystalline aspect. It is rendered completely inactive by thirty minutes' heating up to 60° C. One hour's heating at 55° C. reduces its toxicity. The alkalies, carbonic acid, organic acids, and dilute mineral acids, do not modify it. The concentrated mineral acids destroy its toxicity.

As to the nature of this tetanus poison, Tizzoni and Cattani note numerous similarities with the soluble ferments. In gelatin the tetanus bacillus secretes a peptic ferment which it does not produce in bouillon, and all the influences which modify the tetanus poison destroy also the peptic diastase.*

In their researches on the bacterial poisons, Brieger and Fränkel speak of the toxalbumin of tetanus. This toxic substance was soluble in water. Its parent cultures were developed in sweetened

*Tizzoni and Cattani: Sul Veleno di Tetano (Riforma Medica, 1890, No. 128). Tizzoni and Cattani: Experimentelle Untersuchungen über das Tetanusgift (Archiv f. Exper. Pathol., Bd. xxvii).

bouillon, then filtered through the Chamberland filter, reduced by evaporation in a vacuum, and precipitated by absolute alcohol.* Vaillard and Vincent have obtained in tetanus cultures, made in bouillon and filtered, an extremely active poison, of which $\frac{1}{100}$ and $\frac{1}{1000}$ c.c. sufficed to killed a guinea-pig. They have confirmed most of the foregoing data: thus, for instance, the feeble resistance of the poison to heat, and its inactivity in the digestive tube. Among the new data of their work, I may cite especially the following facts: Direct solar light destroys rapidly, in free air, the toxicity of the filtered liquid. Acidification does not modify its toxic power. Absolute alcohol does not alter the toxic substance; it precipitates it in part, and the product of this precipitation is *tetanigen*. The tetanus poison adheres to certain precipitates which are produced in the liquids in which it is contained: thus it is that the precipitates of phosphate of lime or of alum carry down a part of this active substance, but this precipitation is quite incomplete. Vaillard and Vincent believe that the tetanus poison belongs to the diastases.†

Kitasato has made a detailed study of the tetanus poison. He has determined the exact length of time

*Brieger and Fränkel: Untersuchungen über Bacteriengifte (Bul. Kl. Woch., 1890, Nos. 11 and 12).

†Vaillard et Vincent: Le Poison Tétanique (C. R. de la Société de Biologie, 1890, Nov. 13). Vaillard et Vincent: Contribution à l'Étude du Tetanos (Annales de l'Institut Pasteur, 1891, No. 1).

necessary for the different physical influences (light, heat, etc.), or such chemical influences as acids and alkalies to destroy the tetanus poison. He has found that absolute alcohol added in sufficient quantity not to leave the poison in solution destroys it. In general, Kitasato has not found the means of isolating the tetanus poison from the liquid in which it is contained.*

Here end our sources of knowledge as to the chemical nature of the tetanus poison. As to the mode of its action on the animal organism, we have not made much advancement since the researches of Knud-Faber. We know that the poison reproduces exactly all the symptoms by tetanus in the same way as we obtain them with the bacillus itself. These phenomena of systemic poisoning do not appear immediately after the injection of the toxic liquid, but after a certain time of incubation, which diminishes as the dose or the toxicity of the injected filtered liquid augments.

The morbid symptoms begin, in the case of subcutaneous injection of the liquid, at or near the point of inoculation. The first contractions appear in the muscles nearest the seat of inoculation. Bruschetini has studied the diffusion of the tetanus poison in the animal organism; he finds that it takes place chiefly

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* Kitasato: Experimentelle Untersuchungen über das Tetanusgift, t. x, p. 267.

along the nervous system. He made use of filtered cultures of tetanus.*

Camara Pestana, working with filtered cultures, has carried out the following conclusions:

1st. The absorption of the toxine takes place by the blood.

2d. The lungs, the spleen, the kidneys, but principally the liver, take from the blood the toxic principle, and retain it.

3d. The toxine is not appreciably eliminated by the urine.

4th. Despite the predominence of the neuro-muscular phenomena, no one has yet succeeded in detecting the presence of the toxine in the nervous and muscular tissue, and all the experiments made with these tissues have given negative results.†

The presence of the toxine in the blood of persons affected with tetanus has also been noted by Bruschetini, Kitasato, and Nissen.

The physiological study of the influence of the tetanus poison on the different parts of the neuro-muscular apparatus has not yet been made, and we do not know how this poison acts. Faber has presented

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* Bruschetini: Recherches Préliminaires sur la Diffusion du Poison du Tétanus dans l' Organisme (Annales de Micrographie, 1890, Nov. 20).

† Camara Pestana: Diffusion du Poison du Tétanos dans l'Organisme (C. R. de la Société de Biologie, 1891, June 27).

the hypothesis that it affects the terminal nerve-plates, like curare.

With regard to the subject of immunity from tetanus, there are some important details. At first, all the experimenters arrived at constantly negative results in trying to vaccinate animals against tetanus. At length, Behring and Kitasato announced that they had succeeded in rendering animals immune by vaccination. The animals vaccinated against tetanus exemplified by their behavior the following facts, which were quite new and important:

1. The hares which were vaccinated and resisted the inoculation of the living tetanus bacillus, were also refractory to the injection of the chemical poison produced by this bacillus.

2. This insensibility of the vaccinated animals to the poison is not due to an habituation of their organism to the poison, but to its destruction in their bodies. In fact, the blood, and more particularly the serum of the blood, of the vaccinated hares, when mixed with the tetanus poison, even in small proportion, destroys it. The blood of animals not vaccinated does not possess this antitoxic property.

3. The serum of vaccinated animals possesses not only *in vitro* the antitoxic property, it also exercises it in the bodies of other animals. For the serum of vaccinated hares, when injected in mice, renders them refractory to subsequent inoculation of the bacillus or of the tetanus-poison. It may even cure tetanus in mice already sick.

We see that this work of Behring and Kitasato contains three new and important notions: Vaccination against tetanus; the antitoxic action of the animal humors; and immunization by these humors. These three notions have been controlled and studied since then by many experimenters, and in general have been found perfectly exact. In the following chapter we shall state the results acquired by these studies.*

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*Behring et Kitasato: Ueber das Zustande-Kommen der Diphteric immunität und der Tetanus immunität bei Thieren (Deutsche Medic. Wochenschrift, 1890, No. 49).

CHAPTER X.

THE POISONS OF TETANUS—(Continued).

SUMMARY.—*Vaccination against Tetanus is Obtained by Tizzoni, Cattani, and Vaillard—The Researches of Kitasato; of Behring; of Brieger, Kitasato, and Wassermann—Immunization—The Labors of Tizzoni, Cattani, and Vaillard—The Researches of Ehrlich; of Brieger and Ehrlich; of Brieger and Frank—Application of the Method of Behring and Kitasato to the Treatment of Tetanus in Man.*

We must remark that following the epoch of his publication with Behring, Kitasato did not possess a method of vaccinating animals against tetanus. He had only at his command certain animals which were shown to be fortuitously vaccinated; for a long time he had been able to give only erroneous procedures for this vaccination.

But independently of these researches other experimenters have succeeded in vaccinating animals against tetanus.

Tizzoni and Cattani have vaccinated pigeons and dogs against tetanus by injecting little doses of the living cultures of tetanus. On these vaccinated animals they have been able to confirm the fundamental

propositions of Behring and Kitasato respecting antitoxic reactions and immunization.* Vaillard, in utilizing the method of C. Fränkel (which we shall describe under the head of Diphtheria), has been able to vaccinate hares. The method of Vaillard consists in heating for an hour, at 60° C., filtered cultures of tetanus. The tetanus poison is modified by heat to the point of being unable longer to provoke tetanus even when injected in large doses. But it acquires, by heating, the property of conferring immunity. If we heat the filtered cultures to 65° C., they no longer confer immunity.†

Kitasato‡ described later a process for vaccinating against tetanus. It consists in inoculating hares with the living and virulent cultures of tetanus, mixed with progressively decreasing doses of bichloride of iodine. But the results were first but little favorable, for out of fifteen hares he succeeded in vaccinating only six. Since then, however, the process with ter-chloride of iodine (ICl_3) has been sensibly improved at Koch's Institute by Behring,§ so far as to

*Tizzoni and Cattani: Ueber die Art einem Thiere die Immunität gegen Tetanus zu übertragen (Centralblatt f. Bacteriologie, t. ix, No. 6).

†Vaillard: Immunite contre le Tetanos (C. R. de la Soc. de Biol., 1891, Feb. 21).

‡Kitasato: Experimentelle Untersuchungen über das Tetanusgift (Zeitschrift f. Hygiene, t. x, p. 267).

§Behring: Ueber Immunisirung und Heilung von Versuchtieren beim Tetanus (Zeitschrift f. Hygiene, t. xii, p. 45).

enable us to vaccinate without great danger all the animals under experimentation: mice, hares, sheep, and horses. Still this process makes the animals quite sick for some time. Lastly, Brieger, Kitasato, and Wassermann have found at Koch's Institute a method of vaccination perfectly inoffensive. Resuming the already antiquated researches of Wooldridge on cultures of the microbes in extract of thymus, and following exactly his indications, these authors have made, with the thymus,* culture-media which have given them interesting results with the different microbes, especially with the bacillus of tetanus. These tetanus cultures made in extract of thymus are asporogenous and very slightly toxic. On the other hand, the extract of thymus mixed with a filtered culture of tetanus made in ordinary bouillon, and very toxic, destroys little by little the toxicity of the culture product. In employing these mixtures when two days old in progressively increasing doses, the authors have succeeded in vaccinating without danger and without apparent trouble, animals, such as mice, the most sensitive to tetanus.†

Vaillard has recently come back to the question

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* Wooldridge: Versuche über Schuzimpfung auf Chemischen Wege (Archiv f. Anatomie und Physiologie, Physiologische Abtheilung, 1888, p. 527).

† Brieger, Kitasato, and Wassermann: Ueber Immunität und Giftfestigung (Zeitschrift f. Hygiene, t. xii, p. 1887).

of vaccination against tetanus.* Besides his old method of vaccination by cultures filtered and heated to 60° C., he indicates two new processes: one of these is very nearly like that of Behring and Kitasato, and consists in injecting in animals tetanus-cultures to which iodine solution has been added; the other is identical with that of Tizzoni and Cattani—inoculation with extremely small doses of the virus. Behring has also utilized with success this process of vaccination after dilution.

It will be seen that this question of vaccination has gone through three successive stages. At first, experimenters only succeeded in vaccinating a certain proportion of the animals under trial, while the others died in consequence of the manipulations. Then they obtained more constant results, but the vaccinated animals suffered more or less severely in their general health (local tetanus, fever, and emaciation). At last they found a method both sure and inoffensive (Brieger, Kitasato, and Wassermann). The antitoxic properties of the serum of vaccinated animals were soon found by all the experimenters.

Tizzoni and Cattani have made a special study of the antitoxic substance of serum. They have found that their antitoxine is enfeebled by heating to 65° C. half an hour, and completely destroyed by heating it the same length of time at 68° C. (the

* Vaillard, Annales de l'Institut Pasteur, 1892, No. 4.

temperature of coagulation of the serum). They have found also that this antitoxic substance does not dialyze; that it is destroyed very quickly by hydrochloric acid, by lactic acid in great excess, and by the alkalies. It is precipitated by ammonium sulphate in supersaturation, and also by absolute alcohol. From this latter precipitate it can be extracted by water or by glycerin.*

Vaillard has studied especially the relations between the immunity of animals and the antitoxic property of this serum. He has been led by his researches to deny the possibility of explaining immunity by the antitoxic property of serum. In animals naturally refractory, as, for instance, hens, the serum in the natural state is not antitoxic; it only acquires this property after the injection of a large dose of tetanus poison. Likewise, in the vaccinated animals, one may not find the antitoxic state of the serum. This state only appears as the result of the action of large doses of the soluble products of the tetanus bacillus. The antitoxic action of the serum is only a contingent property of the refractory animals. It cannot, therefore, serve to explain natural or acquired immunity. We must add that Vaillard did not succeed in curing tetanus by the serum of his refractory animals. He found also that the spleen and

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*Tizzoni et Cattani: Ueber die Eigenschaften des Tetanus Antitoxins (Centr. f. Bacteriologie, t. ix, No. 21).

aqueous humor of the vaccinated animals did not possess antitoxic properties. Their muscles, however, possess these properties, at least *in vitro*.*

Apropos of the spleen, we must speak of the researches of Tizzoni and Cattani, who have found that it is impossible to vaccinate against tetanus animals that are deprived of their spleen.

The third thesis of Behring and Kitasato—vaccination and cure by the serum of vaccinated animals—has also been contested. While succeeding in *preventing* the disease by the serum, neither Tizzoni and Cattani, nor Vaillard, succeeded in curing it.

Many other contradictions have been affirmed against the general theory of immunization—contradictions due for the most part to the partisan influence of the old theories of immunization; but all these contradictions have received their quietus, thanks especially to the interesting researches of Ehrlich.† Experimenting with abrine and ricine, two toxic albuminoids of vegetable origin, Ehrlich demonstrated numerically that the immunity of animals against the poison is not an invariable quantity, but may have very different degrees. He demonstrated, moreover, that with the degree of the immunity

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* Vaillard: Property of the Serum of Animals Refractory to Tetanus (C. R. de la Société de Biologie, June 6, 1891).

† Ehrlich, Deutsche Med. Wochenschrift, 1891, Nos. 32 and 44.

acquired by the vaccinated animals varies also the antitoxic and immunizing power of their serum.

Thus were cleared up all the previous difficulties; the experimenters who could not cure, but who vaccinated only with the serum of vaccinated animals, had evidently not obtained in those animals the grade of immunity sufficient for their serum to be curative. In fact, in continuing their experiments, Tizzoni and Cattani, and Vaillard, have perfectly succeeded in curing their tetanic animals with the serum of the vaccinated animals. At the present time, there remains no longer any doubt as to the reality of the third thesis of Behring and Kitasato; we can prevent and we may cure tetanus by the serum of animals vaccinated against this disease.

Erhlich has contributed other interesting documents to this question of the antitoxines. He has seen that if we vaccinate against one of the vegetal toxines pregnant mice, and give them after they have dropped their young the young of non-vaccinated mice to suckle, these little mice become in their turn refractory to the given poison.*

These facts are of the highest importance, for they demonstrate that the immunizing substance is secreted in the milk, and that it is absorbed by the digestive canal. Ehrlich has applied this datum to the infectious diseases.

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* Ehrlich, Zeitschrift f. Hygiene, t. xii, p. 183.

He has been able to prove that the milk secretes antitetanine, and in that condition, being ingested, confers immunity against tetanus. With Brieger he has made other experiments on a large scale. A pregnant goat was by them vaccinated against tetanus, according to the method of Brieger, Kitasato, and Wasserman. Later on, the goat's milk, injected in mice, immunized them perfectly against tetanus.

Behring and Frank have recently published some interesting researches on the properties of the substance which immunizes against tetanus.* They found that the serum of a vaccinated horse kept for two months its immunizing power, although preserved (with a ½-per-cent. solution of phenic acid) in a flask closed with a glass stopper, with no other precaution against the entrance of the air or of microbes. They found also that this immunizing power of serum is not abolished by dilution with distilled water, or by heating it twenty-five minutes at 65° C.—when it begins to coagulate. They insist also on the fact that to cure tetanus when declared, it is necessary to employ stronger doses of the serum than would be required for propyhlaxis.

We must say a few words, in conclusion, about the therapeutic attempts made on man with the immunizing serum. Kitasato was the first to attempt

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* Behring and Frank, Deutsche Medic. Wochenschrift, 1892, No. 21.

to cure tetanus in man by the serum of the vaccinated hare. He employed very feeble doses, and his attempt was not crowned with success. Tizzoni and Cattani, on the contrary, report already seven cases of cure by their antitetanine, prepared with the serum of vaccinated dogs.

More recently, this treatment was attempted without success in two cases of human tetanus in Paris.* It is evident that there have as yet been too few cases to warrant speaking for or against the method of Behring and Kitasato. But the brilliant results of experimentation leave no doubt that success on man is only a question of method and of doses.

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*Annales de l'Institut Pasteur, 1892, No. 4.

CHAPTER XI.

THE POISONS OF DIPHTHERIA.

SUMMARY.—*The Researches of Roux and Yersin; of Löffler—C. Fränkel Succeeds in Vaccinating Animals against Diphtheria—The Researches of Behring and His Discovery of Immunization—The Difficulties Not Yet Surmounted of Diphtheritic Vaccination.*

In filtering the diphtheritic cultures through the Chamberland filter, Roux and Yersin obtained toxic liquids. These filtered cultures injected in animals produce the same series of symptoms as the living diphtheria bacillus. In guinea-pigs these filtered cultures generally cause the following lesions: At the place of inoculation is found an exudative œdema; the lymphatic glands are congested; the small intestine, the lungs, and the suprarenal capsules are engorged; and the pleura contains a serous effusion. The systemic intoxication of hares is characterized especially by diarrhœa and fatty degeneration of the liver. In smaller doses which kill only after a long time, the poison of the filtered cultures may provoke in animals the different paralyses which are so frequent in diphtheria in man.

The toxic substance is completely destroyed by heating for ten minutes up to 100° C.*

In another memoir, Roux and Yersin have studied at greater length the properties of this diphtheritic poison, which they have classed among the diastases † We shall not enter farther into the discussion as to the chemical nature of this poison—a discussion which is set forth at length with all its bibliographical details in Chapter VI.

Löffler has confirmed the existence of the diphtheritic poison. He extracted it by means of glycerin from cultures made on hashed meat.‡

The resistance of the diphtheria poison to the different means of destruction has been but little studied. Heat above 60° C. destroys it, or, as we believe, modifies it, leaving the cachectizing poison uninjured. The same decomposition is obtained by the action of the peptic diastases, such as trypsin and pepsin. Contact with alcohol modifies also the diphtheritic poison. The different oxidizing agents, as potassium permanganate, destroy it completely. The reducing agents, such as HS_2, have no action on it.

Roux and Yersin have completely failed in their

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*Roux and Yersin, Annales de l'Inst. Pasteur, 1888, No. 12.

†Roux and Yersin: Study of Diphtheria (Annales de l'Inst. Pasteur, 1889, p. 273).

‡Löffler, Deutsche Medic. Wochenschrift, 1889, Nos. 5 and 6.

attempts to habituate or to vaccinate animals against the diphtheritic poison. C. Fränkel has been more successful. In heating cultures of diphtheria at 70° C., he has succeeded in depriving them in great part of their toxicity, while not injuring their vaccinal power. He has found that the heating of diphtheritic cultures three weeks old should be made at the temperature of from 60° to 70° C. If this temperature is exceeded, you obtain no longer vaccinant effects, and you even produce a slow intoxication of the animals. With cultures heated to 70° C., and inoculated in the dose of from 10 to 20 cubic centimeters under the skin of guinea-pigs, immunity is conferred at the end of two weeks.*

Shortly after Fränkel's communication, appeared the memoir of Behring on the same subject. Behring gives five different processes of vaccinating animals against diphtheria:

1. With the sterilized cultures, as in the method of Fränkel.

2. With diphtheritic cultures to which has been added trichloride of iodine.

3. By the pleuritic exudation of guinea-pigs which have died of diphtheria.

4. By the inoculation of animals with the virulent diphtheritic bacillus followed by their treatment by trichloride of iodine in subcutaneous injection.

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* *Vide* Gamaleïa, in C. R. de la Soc. de Biologie, Feb. 20, 1892; also C. Fränkel, Berlin. Klin. Woch., 1890, No 49.

5. By oxygenated water, which has the property of vaccinating the animals against diphtheria, just as trichloride of iodine vaccinates them against tetanus.

The great number of processes which were only touched upon in the memoir of Behring made it plain that this author did not possess a good method of vaccination against diphtheria.* And his subsequent publications only went to confirm this conviction.

Proskauer and Wassermann have attempted to vaccinate against diphtheria by the poisons subjected to heat, but with constantly negative results.†

Zimmer, at the laboratory of C. Fränkel, has verified the exactitude of the assertions of Behring. He has noted, in general, that a more or less complete vaccination may result from the employment of these different processes, but that no one of them is certain. With most of the processes of Behring the immunity of the animals is the exception, and an ultimate death is the rule.

Only one of the methods of Behring should be signally excepted: it is that of the injection in the animals of diphtheritic cultures attenuated by the addition of trichloride of iodine. This process has given Zimmer positive results.‡ It is far, however,

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* Behring, Deutsche Medic. Woch., 1890, No. 50.

† Proskauer and Wassermann, Deutsche Med. Woch., 1891, No. 17.

‡ Zimmer, Deutsche Medic. Woch., 1892, No. 16.

from constituting a definitive method of vaccination, for Behring himself does not trust it; in his most recent work on this subject he describes as his method of anti-diphteritic vaccination certain extremely complicated processes. He injects successively diphtheritic cultures heated to 90°, 80°, and 70° C.; then mixtures, at different degrees of strength, of cultures with trichloride of iodine. Despite this long and complicated preparation, you will note in his experiments that most of his vaccinated animals die of diphtheria. Moreover, Behring himself acknowledges that he does not possess a satisfactory method of vaccination.

We shall see further on, what enormous interest is attached to this problem of how to find a sure method. The principles which guide Behring in his vaccination procedures are the following:

To give immunity to receptive animals, and to augment immunity in animals which already possess it in a certain degree, the vaccinal injections ought to be followed by a reaction of the organism. This reaction is general and local.* Behring does not indicate in what the first consists; as to the local reaction, it is a tumor more or less circumscribed. If the vaccinal reaction is *nil*, the vaccinal injection will

* Behring and Wernicke, Zeitschrift für Hygiene, t. xii, p. 10. Also, Gamaleïa's articles in Ann. de l'Inst. Pasteur, 1888, No. 10, and Centralblatt f. Bacteriologie, 1888, t. iv, p. 161.

not give any immunity, or will not augment the pre-existing immunity. If the reaction is excessive, the vaccinal injection leads to an opposite effect; it augments the receptivity of the animal to diphtheria. If the reaction is moderate, the animals acquire a certain degree of immunity, but only at the end of a certain time—several weeks or several months. According to this exposition, you can see with what difficulty and slowness vaccination is made by the procedures of Behring.

Consequently, it is with reason that the authors of a recent work on the subject—Brieger, Kitasato, and Wassermann—declare that there does not thus far exist any process which enables us to vaccinate with certainty against diphtheria. In making use of Wooldridge's method, they give, in their turn, a new procedure: they cultivate the diphtheritic bacillus in extract of thymus, and then heat this culture (after C. Fränkel) between 60° and 70° C. for fifteen minutes. With the vaccine prepared in this way, they have obtained some positive results. But this mixed procedure is not more sure.*

Although we do not yet possess a satisfactory method of vaccination against diphtheria, the fact of the possibility of this vaccination is sufficiently established.

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*Breiger, Kitasato, and Wassermann, Zeitschrift für Hygiene, t. xii, p. 137. Also, Behring and Kitasato, Deutsche Medic. Wochen., 1890, No. 49.

The study of these vaccinated animals has led Behring and Kitasato to results extremely interesting.

Behring* has noted—and this fact has been confirmed by other observers—that the inoculation of the diphtheritic bacillus in vaccinated animals is followed by the formation of a necrotic patch under which the bacillus long lives in a state of activity. It is not, we perceive, destroyed in the vaccinated organism. It does not even seem to have lost its virulence, for its subsequent inoculation in animals not vaccinated provokes in them typical diphtheria. As, however, it remains inoffensive to the vaccinated organism which gives it harbor, Behring supposed that the latter possesses the power of destroying its poison.

We have seen in the history of the tetanus poison how this supposition was confirmed by Behring and Kitasato.

For diphtheria, as for tetanus, these authors found in the serum of vaccinated animals certain remarkable antitoxic properties. The serum of certain animals vaccinated against diphtheria — guinea-pigs, hares, sheep—has the power to destroy *in vitro* the diphtheritic poison; it may consequently prevent and cure diphtheria in animals in which it is injected in sufficient quantity.

If all these researches have not yet led to any

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* Behring: Communication to the Congress of Hygiene. London, 1891.

good practical result in the cure of diphtheria in man, this is due solely to the difficulty of procuring the serum of vaccinated animals in great quantity; and this difficulty depends on the absence of a good method of vaccination. It is for this reason that we have here dwelt at such length on the question of vaccination against diphtheria.

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CHAPTER XII.

THE POISONS OF CHOLERA AND OF THE AVICIDE VIBRIO.

SUMMARY.—*The Researches of the Author on the Poisons of Cholera and of the Avicide Vibrio—The Researches of Hernandez and Brühl, of Niessen and Behring, and of Zasslein.*

The history of the development of our sources of information respecting the poisons of cholera, shows in a typical manner the struggle of the different conceptions concerning the chemical nature of the microbian poisons. In order to explain cholera, we have seen successively advanced such considerations as the products of the metabolism of the Indian vibrio, the diastases which it secretes, and, lastly, the action of the microbe by its own substance.* Cantani was the first to indicate that the cholera poison may be neither a diastase nor a ptomaïne, but the cholera vibrio itself. This he has not proved.†

I have myself found the two cholera poisons: the primary poison and the modified poison. The first,

* Gamaleïa: Experimental Researches on the Poisons of Cholera (Arch. de Méd. Exper., March 1, 1892).

† Cantani, Deutsche Medic. Wochen., 1886, No. 45.

which we believe to be a nucleo-albumin, has the property of provoking a systemic intoxication exceedingly like the symptoms of Asiatic cholera. In hares this intoxication manifests itself chiefly by a prolonged and violent diarrhœa; in dogs the vomitings predominate, and may last several hours. In all cases the poison determines a violent lesion of the gastro-intestinal tube.

This poison is extremely fragile. It is destroyed by heat above 60° C., by alcohol, and by the strong alkalies. It is carried down by the different precipitates which may be produced in the cholera cultures; for example, by that which is formed by acetate of lead. It may be extracted from them by alkalinized water.

What is the mode of action of this poison? By some of its reactions it resembles the soluble ferments. We know also that the cholera vibrio may produce the different diastases. It may be asked if it is not these diastases which are toxic in the cultures of cholera? Some think that the excessive production of liquid in cholera stools is the result of a hypersecretion of the intestinal glands (Cohnheim), and we know that the substances secreted by the glands stimulate their activity if injected into the blood. In studying the toxic action of the cultures of different microbe producers of diastases, we find, in fact, that they all have as a common character a certain diarrhœic action, though this is most marked in

the cholera cultures. Even certain samples of pancreatin, in our experiments produced diarrhœa in hares. But the results with this latter substance have not been constant, and we have at least succeeded in procuring some trypsin which is extremely active as a peptic diastase and which has no diarrhœic action. Many other ferments which we have studied in this connection were also devoid of the property of provoking diarrhœa.

We must conclude that the diarrhœic poison of cholera does not belong to the category of these ferments.

The diarrhœic action is very general in the class of microbian poisons, but it is due to another mechanism than that indicated above.

We have found still another cholera poison, which is very stable. It belongs to the class of our modified poisons, or nucleines, which Buchner has denominated proteins or alkali-albumins. This poison has no other interest than that of being associated with the vaccinal substance of cholera. But we shall not here set forth the details of cholera vaccination, which is still under controversy. There exists a microbe which resembles in all respects that of cholera. It is the avicide vibrio, which I discovered at Odessa. Chemical vaccination with this microbe is at present the best known of all the vaccinations. As, besides, it has many analogies with cholera vaccinations, we shall venture to say a few words about it.

The vaccinal substance or substances which are potent against the avicide vibrio are also associated with the nucleine or modified poison of the vibrio. They may be isolated in two ways. First, if you subject to distillation in a vacuum the vibrionic cultures, the substances which pass over have a manifest vaccinal action, while the toxic nucleine remains in the residue. Now Brühl has found that if we precipitate the toxic substance by acetate of lead, the filtrate vaccinates perfectly. The vaccinal action of the volatile products would lead one to think that the vaccine is a ptomaïne, but the ordinary methods for the isolation of the ptomaïnes have resulted only in inactive substances (Brühl). Alcohol especially seems to enfeeble the vaccinal properties of the vibrionic cultures.

The study of the serum of animals vaccinated against either the avicide vibrio or the cholera vibrio reveals certain remarkable peculiarities. While the serum of ordinary guinea-pigs favors the growth of the avicide and cholera vibrios, the serum of guinea-pigs vaccinated against these diseases acquires the property of completely destroying these microbes. We have no need of insisting upon the bearing of these facts on the true solution of the problem of immunity.

Still another fact of considerable theoretical importance has been found in connection with the diseases caused by these two vibrios. Guinea-pigs which

are vaccinated against the avicide or cholera vibrio are not more resistant to the vaccinal poison than non-vaccinated guinea-pigs. This fact is of fundamental interest in reference to the entire mechanism of vaccination.

Denied at first by certain bacteriologists, it was later affirmed by the same, and adduced in opposition to the great principles established for tetanus and diphtheria. We have seen that in these two diseases the animals which are vaccinated against the living microbe become also refractory to the microbian poisons.

Cholera and the vibrionic septicæmia seemed to be an exception to this rule, for in these two diseases the animals succumb to the same dose of the poison as the control animals. But this exception is only apparent, and it disappears when we recognize the distinction between the primitive and the modified poisons of bacteria.

Towards the primitive poisons of cholera and of septicæmia, the vaccinated animals are quite as refractory as is the case with diphtheria and tetanus. They are not refractory towards the modified poisons; but as these latter are artificial products, this has nothing to do with the theories of immunity.

With regard to the mechanism of vaccination, it may appear strange that the modified poisons to which the animal is not habituated render it refractory to the primitive poisons and to the bacteria.

Here also we must make a distinction. The vaccinal substance is not the modified poison, for it may be separated from it. Not being toxic, it does not vaccinate the animals by the fact of habituation, but probably by forming in their bodies a compound which has antitoxic properties.

CHAPTER XIII.

THE POISONS OF TUBERCULOSIS.

SUMMARY.—*The Researches of Koch, of Maffuci, of Prudden and Hodenpyl, of Straus and the Author, of Grancher and Ledoux-Lebard—The Tuberculin of Koch, and the Researches which it has Provoked—The Toxomucin of Weyl.*

The tuberculous poisons have only lately been found. Bacteriologists were long and unsuccessfully searching for the toxic substances in the cultures of Koch's bacillus, and they have only found them by applying directly to the bacillus itself.

Koch has seen that the bacillus tuberculosis when dead provokes by subcutaneous injection the formation of abscesses in animals. Moreover, he has obtained from the bodies of the bacilli a substance called tuberculin, which has long been believed to exercise a specific action on the tuberculous.*

Maffuci has found a cachectizing influence in tuberculous cultures dead by age or killed by discontinuous sterilization.†

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* Koch, Deutsche Medic. Wochenschrift, Jan. 18, 1891.

† Maffuci, Centralblatt f. Allgemeine Pathol., Dec. 15, 1890.

Prudden and Hodenpyl have seen that the bacilli when boiled and well washed may give rise to the formation of curable nodular lesions.*

Straus and I have shown that the cadavers of tubercle bacilli provoke in different animals a disease which in its course and its lesions is extremely like tuberbulosis produced by living bacilli. This disease, which Grancher and Ledoux-Lebard have propossd to call necro-tuberculosis,† is characterized by the development in inoculated animals of a great sensitiveness towards a new inoculation of tuberculosis. This predisposition manifests itself by rapid death after re-inoculation. The inoculation of dead bacilli produces such changes in the animal organism that the animals die very speedily after a new injection of tuberculosis, an injection which produces only tardy effects in healthy animals.

Another symptom of necrotuberculosis, but much more apparent, is the continuous and progressive emaciation of the animals. They may lose as much as one-half of their initial weight. They die in a profound cachexia. At the autopsy we find characteristic lesions.

The different organs, and especially the lungs, in

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* Prudden and Hodenpyl, New York Medical Journal, June 6 and June 20, 1891.

† Straus and Gamaleïa, Archives de Médecine Expérimentale, 1891, No. 6. Grancher and Ledoux-Lebard, Arch. de Méd. Expér., 1892, No. 1.

the case of intravenous injection of dead bacilli, are strewn with granulations formed of epithelioid and embryonal cells, and containing tubercle bacilli. The volume of the nodules which may be thus produced in the different organs depends almost exclusively on the size of the mass of cadavers which has been inoculated. It is very interesting to note that the animal organism cannot dislocate the bacterial colonies which are inoculated into it.

When once the existence of the tuberculous poison, and its presence in the cadavers of the bacilli, was established, it was necessary to seek to determine the toxic substance and to isolate it from the bacilli.

There were evidently different means for proceeding to this extraction. By alcohol and ether Hammerschlag has extracted from the bacilli, in addition to lecithin and fat, a toxic substance which kills animals with convulsions.* Later, Zuelzer also succeeded in finding a convulsivant ptomaïne in tuberculous cultures.†

Much more interesting was Koch's discovery. By means of glycerin-water at a boiling temperature and in a neutral medium, Koch has extracted from tubercle bacilli an albuminoid substance called tuberculin, which provokes in tuberculous animals fever

*Hammerschlag, Correspondenzblatt für Schweizerärzte, Oct. 15, 1888, and Centralblatt f. Klin. Med., Jan. 1, 1891.

†Zuelzer, Berlin. Klin. Wochen., Jan. 26, 1891.

and inflammation of the bacillary foci. Koch has studied in detail the many very interesting properties of this tuberculin.* Tuberculin dissolved in glycerin-water is a very stable body which supports a heat of 160° C. It is easily precipitable by alcohol.

But if we attempt to purify it and to free it from glycerin and salts, its properties are modified. Aqueous solutions of purified tuberculin are very unstable in absence of glycerin. They easily give a precipitate more or less abundant of a substance, insoluble in water but soluble in alkalies, which preserves the toxic action of the tuberculin. In absence of salts, the purified tuberculin is no longer precipitated by absolute alcohol.

The purified tuberculin has all the reactions of albuminoid substances. It gives a precipitate by acetic acid which is soluble in excess of the reagent. It contains a great quantity of phosphorus. But it is evidently not a pure substance, for it leaves from 14 to 21 per cent. of ashes, and then its mode of preparation by the concentration of cultures made in bouillon adds to the extract of bacillary bodies all the substances pre-existing in the bouillon.

Hunter † has endeavored to advance still farther our knowledge of tuberculin. Unfortunately he also

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* Koch, Deutsche Medic. Wochen., 1891, No. 43. Gamaleïa, Arch. de Méd. Expér., 1891, No. 2.

† British Medical Journal, July 28, 1891.

made use, in his chemical researches, of the crude tuberculin of Koch, prepared by means of culture bouillons, instead of taking the bacilli themselves, washed and cleaned. In this crude tuberculin Hunter found the four albumoses of Kuehne and Chittenden. It is to be regretted that he did not attempt to find them in the primitive bouillon of the cultures.

Hunter has also compared the differences in the action on the tuberculous man and animal of the different modified tuberculins. The modification A, which is the precipitate obtained by absolute alcohol, has the property of producing the local reaction with very little fever. The modification C, which contains the substances not precipitated by absolute alcohol, produces chiefly a high fever. The modification B, which is the precipitate obtained by supersaturation of the tuberculin with ammonium sulphate, possesses the power of provoking the local and salutary reaction without constitutional disorders. Lastly, the modification CB has all the curative virtues of Koch's tuberculin, without possessing the disadvantages—either the local inflammatory or the general febrile reaction. This happy modification, CB, is obtained by getting rid, by absolute alcohol, of most of the albumins, and by the dialysis of other injurious substances. These results of Hunter are founded on an altogether insufficient number of experiments. Furthermore, according to all the other experimenters, and particularly Pfuhl, the collaborator of Koch, tuber-

culin has not any curative properties, especially if you prevent the local reactions.*

The tuberculocidin of Klebs, prepared by means of tuberculin, is entitled to no greater confidence.

As to the chemical nature of tuberculin, we cannot pronounce with certainty until we have a purer preparation. There is no doubt that it belongs to our class of modified poisons, which we suppose to be nucleines or nucleinic acids. It is evident, also, that in Koch's tuberculin is not found the primary tuberculous poison. The action of tuberculin does not explain the effects produced by the dead bacilli. This action is not characteristic of tuberculin, for it is also provoked by many other bacterian extracts. The effects proper to the tuberculous poison contained in the microbian cadavers, the predisposition which it creates, and the suppurative and caseous lesions, cannot be produced by tuberculin.

More important in this respect is the substance which Weyl has extracted from tubercle bacilli by means of a solution of caustic soda. This substance, insoluble in acetic acid, is classed by Weyl among the mucins, but it does not form a reducing substance, and it contains phosphorus. It may be nucleo-albumin in the sense of Kossel and Hammersten.

This mucin of Weyl has a toxic action. By subcutaneous injection it produces in mice and

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* Pfuhl, Zeitschrift für Hygiene, t. xi, page 241.

guinea-pigs a necrosis of the skin. But the toxicological study of Weyl is too incomplete. We cannot but suppose that he had under observation the actual primary tuberculous poison, that which produces the local lesions of tuberculosis.*

* Weyl, Deutsche Medic. Wochen., Jan. 12, 1891.

CHAPTER XIV.

THE POISONS OF CHARBON AND OF GLANDERS.

SUMMARY.—*Insufficiency of our Toxicological Knowledge respecting Charbon and Glanders—Different Writings on the Subject.*

Charbon (splenic fever, wool-sorters' disease, deadly anthrax), which once had so great a doctrinal importance as to guide the first steps of students in bacteriology, has not preserved this eminent place in toxicological researches.

Toussaint believed that he could confer immunity on sheep and young dogs by means of anthracoid blood freed from living bacteria by filtration or by heating to 55° C., and even by antiseptics.

Chauveau brought powerful arguments in favor of this chemical vaccination.*

After having, with Pasteur, denied its reality, Chamberland and Roux published later some experiments which showed that it is in fact possible to vaccinate sheep against charbon by means of soluble substances.†

*Annales de l'Institut Pasteur, 1888, No. 2.

† Chamberland and Roux, Ann. de l'Institut Pasteur, 1888, No. 7.

Already, moreover, before these experiments, this possibility had been demonstrated by Wooldridge. He found that anthracoid cultures made in a solution of the substance which he called *tissue-fibrinogen*,* might confer immunity on hares if injected subcutaneously, when freed from living microbes by ebullition or by filtration. What is surprising in the experiments of Wooldridge is that his substance conferred immunity not only when it was injected long before the injection of the living and virulent microbe, but also in the case of its simultaneous injection with the microbe. Wooldridge thus furnishes the first instance of the experimental cure of an infection by means of vaccination.†

The interesting researches of Wooldridge, interrupted by the premature death of this young *savant*, have only recently been taken up again by Brieger, Kitasato, and Wassermann. But what is a little singular, the authors having very well succeeded in preparing by the method of Wooldridge chemical vaccines against the different pathogenic microbes, have been less fortunate with charbon. Perhaps

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* Wooldridge, Arch. f. Anatomie und Physiologie, Physiologische Abtheilung, 1888, p. 527. Wooldridge's first publications date from 1887.

† The fibrinogenous substances of Wooldridge are compounds between the albuminoids and lecithin. He extracted them from different fresh organs, and especially from the thymus and testicles of animals.

these men have not been sufficiently persevering in this direction, having the old prejudice as to the radical distinction between the toxic diseases (tetanus, cholera, diphtheria, typhoid fever) on the one hand, and the septicæmias (charbon, rouget of swine, etc.) on the other.*

Wooldridge's experiments on charbon have also been continued by Hankin and Martin.†

Hankin has cultivated the anthrax bacillus in Liebig's beef-extract with the addition of fibrin. From these cultures he has isolated, by precipitating them with alcohol, an albuminoid substance which he calls albumose. This albumose has the property of vaccinating against charbon, and even of curing this disease.

We must, however, immediately add that Petermann, who has repeated exactly the experiments of Hankin, has arrived at entirely negative results. Before Petermann, Landi also obtained only negative results. Martin‡ has cultivated the anthrax bacillus in a solution of pure alkali-albumin. He has studied in detail all the chemical products which form in cul-

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*Brieger, Kitasato, and Wassermann, Zeitschrift f. Hygiene, t. xii, fasc. 2.

†Hankin, British Medical Journal, Oct. 12, 1889, p. 810.

‡Sidney Martin, Proceedings of the Royal Society, May, 1890. Annual Report of the Local Government Board, London, 1889–1890, p. 235.

ture-broths. In accord with Kuehne, he has found the three albumoses and the peptone which result from the digestion of albuminoids by the proteolytic ferments. He has also found an organic base. The albumoses and the base are all toxic, especially the latter. A toxic ptomaïne was long since isolated from anthracoid cultures by Hoffa,* who employed three different methods for its extraction, and who cultivated the anthrax bacterium on meat, or on eggs mixed with bouillon. The ptomaïne of Hoffa is, however, very little toxic like that of Martin.

Lando Landi † has made some very interesting researches on the substances produced by the anthracoid bacterium. He has found a base toxic to mice, of the carbopyridic series, and some peculiar albumoses, which may be obtained in a crystalline state; but his attempts to vaccinate animals with all these products have not succeeded.

Lastly, Christmas‡ seems to have obtained some positive results. He vaccinates hares against charbon by two processes: First, by the diseased anthracoid organs crushed and emulsified in water, in which the bacteria are killed by eucalyptus essence, and the products then filtered through paper. In his

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* Hoffa: Die Natur des Melzbrand Giftes. Wiesbaden, 1886.

† Lando Landi, C. R. de la Soc. de Biologie, July 25, 1892.

‡ Christmas, Ann. de l' Inst. Pasteur, 1891, p. 487.

second process he makes anthrax cultures in a medium composed of yolks of eggs, egg albumin, and veal broth feebly alkaline, in equal parts. The bacilli grow very well in this medium, but do not form spores. After five or six days of incubation at 30° C., the cultures are diluted with water and passed through the Chamberland filter. The filtered liquid possesses feeble vaccinal properties. If the cultures are prolonged beyond sixty-seven days, they become very toxic and not at all vaccinant. These anthracoid poisons have not been studied by Christmas.

To sum up, we see that despite a considerable number of researches the anthracoid poisons still remain almost entirely unknown to us, and the little that we do know is not yet established with certainty.

The poisons of glanders were first studied by Finger.* He found that boiled cultures of the glanders bacillus are toxic; but the phenomena which they produce have no similarity to the symptoms of farcy-infection. He has succeeded in some exceptional cases in conferring immunity on hares against the living microbe by means of these boiled cultures. Later, this question was again taken up by Bromberg,† who has seen that even at 120° C. the toxic properties

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* Finger, Ziegler's Beiträge zur Path. Anatom., 1889, t. vi, p. 373.

† Bromberg, C. R. des Travaux de l'Institut Véterinaire de Kharkow, t. iii, 1889.

of the cultures of the glanders bacilli are not completely destroyed.

Since then, the poisons of glanders have been only studied from the special point of view of malleine applied to the diagnosis of latent glanders, as we have before seen (see page 78).

It is thus seen that our sources of knowledge respecting the poisons of glanders are scant.

CHAPTER XV.

PRACTICAL RESULTS OBTAINED BY THE STUDY OF MICROBIAN TOXICOLOGY.

SUMMARY.—*Rapid Indications respecting the Other Bacterial Poisons—Immunization as a Result of Toxicological Studies.*

We are obliged to stop here in our study of the microbe posons. We must, however, before closing, enumerate some of the special treatises on the subject which the student would do well to consult.

First, the memoirs of Charrin and of Bouchard's laboratory on the pyocyanic poisons;* those of Monfredi and Traversa† and Roger on the poisons of erysipelas;‡ of Chamberland and Roux on the septic poison and that of symptomatic charbon;§ those of Arloing on the poison of the bacillus heminecrobio-

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* Charrin: The Pyocyanic Disease. Paris, 1889. Also his opening article in the new Traité de Médecine, tome i.

† Monfredi and Traversa, in Giorn. Intern. Science Mediche, 1888.

‡ Roger, C. R. de la Soc. de Biol., July 4, 1891.

§ Chamberland and Roux, Ann. de l'Institut Pasteur, 1887, No. 12. Roux, Ann. de l'Institut Pasteur, 1888, No 2.

phylus;* those of Selander on hog-cholera;† those of Hericourt and Rechit and Courmont and Dor on the bacillus of the tuberculosis of birds.‡ We must also cite the researches of Schiff and of his successors who have endeavored to solve the problem proposed by Stich (see page 5).§ But we hasten to abridge all these details, which are very insignificant in comparison with the great principle of which it remains for us to to speak. We refer to immunization.

Thanks to the impetus which has been given the last few years to the researches of microbian toxicology, science has been able to discover and establish definitively on solid bases a principle which is likely to be of inestimable value in medicine.

According to the doctrine of immunization, it is in our power to prevent and cure infectious diseases by the serum of animals refractory and prepared.‖ We have already seen how immunization is realized in

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* Arloing: Les Virus. Paris, 1891.

† Selander: Contribution to the Study of Swine-pest (Ann. de l'Institut Pasteur, 1890, No. 9).

‡ Hericourt and Richet, C. R. de la Soc. de Biol., 1891, p. 470. Courmont and Dor, Arch. de Méd. Expér., 1891, No. 6.

§ Bouchard: Leçons sur les Auto-intoxications. Paris, 1879. Roger: The Action of the Liver on Poisons. Paris, 1886.

‖ This preparation, we may remind the reader, is effected by saturating these refractory animals with the toxic soluble or vaccinant products of the corresponding pathogenic microbes.

the case of tetanus. It has been applied to yet many other diseases. First it has been established for fibrinous pneumonia by the labors of Emmerich and Fovitsky and the two Klemperers.* It has been confirmed by the researches of Archaroff.† Klemperer has given the results of the application of immunization in the treatment of forty cases of pneumonia in man.‡ Immunization has also been applied to the *rouget* of hogs, to the septicæmia of mice, to the disease caused by Friedlander's microbe, and the pyocyanic disease.§ More recently still, a way has been found for giving immunity against typhoid fever, by Brieger, Kitasato, and Wassermann,‖ and for robine by Ehrlich.¶ Seeing the great number of immunizations which have been discovered in the short space of about eighteen months, one might almost be warranted in concluding that immunization is a general principle, and that, consequently, all the infectious diseases are curable by this method. On the other hand, the researches of Ehr-

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*Emmerich and Fovitsky, Münch. Medic. Woch., August 11, 1891. G. and F. Klemperer, Berlin. Klin. Woch., August 24 and 31, 1891.

†Arch. de Méd. Expér., 1892, No. 4.

‡Berlin. Klin. Woch., May, 1892.

§Klemperer, Berlin. Klin. Woch., May, 1892. See also Münch. Medic. Woch., 1892, Nos. 5 and 6.

‖Brieger, Kitasato, and Wassermann, Zeitsch. f. Hygiene, t. xii, fasc. 2.

¶P. Ehrlich, *loc. cit.*

lich, who applies immunization against the poisons of plants, as abrine, ricine, and robine, demonstrate peremptorily that it is against the soluble poisons principally that immunization acts, and that by neutralizing the soluble poison one becomes master of the infection. And this gives support still further to the principal thesis of my book: that the pathogenic microbes are injurious only by their poisons—that infection is only intoxication by the bacterial poisons.

Moreover, it was in the experiments with the poisons of tetanus and diphtheria that immunization was discovered. And it seems a little strange that the discovery was not sooner made, for the studies on the microbian poisons are less complex than the researches on living bacteria. Thanks to the relative simplicity of its problems, microbian toxicology has been able to free itself of the first of the clogs of preconceived ideas which impede and obstruct the other domains of bacteriology.

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